Paula Sofia Fonseca Nabais

Mestre em Conservação e Restauro, especialidade em
Ciências da Conservação

Identity and connections within medieval heritage: color in the illuminated manuscript through the eyes of the molecular sciences and humanities

Dissertação para obtenção do Grau de Doutor em
Conservação e Restauro, especialidade em Ciências da Conservação

Orientador: Prof. Doutora Maria João Seixas de Melo, FCT-NOVA
Co-orientadores: Prof.Doutora Maria Adelaide Miranda, FCSH-NOVA
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FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

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IDENTITY AND CONNECTIONS WITHIN MEDIEVAL HERITAGE: COLOR IN THE ILLUMINATED MANUSCRIPT THROUGH THE EYES OF THE MOLECULAR SCIENCES AND HUMANITIES

Acknowledgements

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Lastly, I dedicate this thesis to my grandfather. Thank you.

Resumo

A caracterização e identificação de corantes orgânicos é um dos principais desafios das Ciências da Conservação. Nesta tese, as potencialidades da microespectrofluorimetria aliada à quimiometria, foram exploradas, permitindo uma identificação do corante, mas também um conhecimento mais aprofundado sobre a produção destas cores. A microespectrofluorimetria no visível permite a aquisição simultânea de espectros de emissão e excitação, combinando elevada sensibilidade e seletividade com uma boa resolução espacial e a possibilidade de análise em camadas, facilitando a identificação mais precisa de corantes e lacas. As especificidades das formulações destes materiais podem fornecer informações sobre particularidades cronológicas e de localização, permitindo uma melhor compreensão da produção dos materiais dos artistas.

A ***primeira parte*** desta tese foca-se no desenvolvimento de abordagem quimiométrica aplicadas a *i)* uma base de dados de reproduções históricas de quatro corantes vermelhos naturais, pau-brasil, goma-laca, cochinilha e quermes, usados durante a Idade Média; *ii)* dados adquiridos em obras de arte, abordando a dificuldade de análise de amostras originais. O primeiro confirmou o potencial da microespectrofluorimetria na caracterização da formulação da tinta; enquanto o segundo explorou as especificidades das cores "originais" e a eficácia desta metodologia para explorar semelhanças entre tintas envelhecidas naturalmente.

Este projeto comprovou a capacidade da microespectrofluorimetria como uma técnica poderosa para a identificação de corantes e lacas. A base de dados de reproduções permitiu identificar as principais receitas das lacas de cochinilha da Winsor & Newton do século XIX. A base de dados de obras de arte permitiu compreender melhor as especificidades de receitas e pela primeira vez foi possível identificar a mistura de goma laca e pau-brasil, em manuscritos do *scriptorium* de Alcobaça.

Numa ***segunda parte***, esta metodologia foi testada em dois casos de estudo: o Cancioneiro da Ajuda e um grupo de manuscritos islâmicos. O primeiro, o mais antigo dos cancioneiros galego-portugueses que chegaram aos nossos dias, é um manuscrito inacabado, do qual ainda não sabemos as circunstâncias da sua produção, ou a razão pela qual nunca foi terminado. Uma abordagem multianalítica combinada com a metodologia desenvolvida nesta tese permitiu a caracterização da paleta molecular. A construção das cores e a riqueza da paleta cromática demonstra o desejo e os recursos para produzir um manuscrito luxuoso. A metodologia permitiu propor uma data de produção para o Cancioneiro da Ajuda, onde a presença de pau-brasil e de ouro musivo indica uma data em torno do século XIV, enquanto o uso de ouropigmento sugere uma produção do século XIII.

O segundo caso de estudo é um grupo de manuscritos islâmicos (séculos XII – XV), provenientes de Tombuctu, Mali, que, devido ao seu resgate e conservação, criaram a oportunidade de estudar os seus materiais e técnicas. Pela primeira vez, a riqueza e especificidade das formulações das cores foi abordada. Foi possível fornecer uma caracterização molecular inequívoca do cromóforo principal, goma-laca, mas também atribuir uma receita específica para a produção do corante. A comparação com a base de dados de obras de arte mostrou semelhanças com as formulações de corantes

encontrados nas iluminuras medievais portuguesas. A caracterização completa dos materiais da cor revelou também degradação substancial do ligante. Esta caracterização da paleta molecular permitirá tomadas de decisão mais informadas durante o processo de conservação e restauro destes manuscritos.

A sinergia entre a abordagem multi-analítica desenvolvida para a análise de manuscritos medievais e a nova metodologia para o estudo de corantes orgânicos foi importante na análise de ambos os casos de estudo. A microespectrofluorimetria, bem como a expertise na caracterização de obras de arte, permitiu aprofundar o conhecimento sobre a construção das cores, muito além da identificação do cromóforo.

Palavras-chave: Manuscritos medievais, Corantes, Lacas, Fluorimetria, Cancioneiro da Ajuda, Manuscritos islâmicos.

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Abstract

The characterization and identification of organic dyes is still a challenge within the field of Conservation Science. By exploring the potentialities of microspectrofluorimetry combined with chemometrics, this doctoral project provides the identification of red organic colorants and a comprehensive knowledge of the making of medieval paints. Microspectrofluorimetry (in the visible) allows the simultaneous acquisition of excitation and emission spectra, offering high sensitivity and selectivity combined with good spatial resolution and the possibility of in-depth profiling, which facilitates an accurate identification of dyes and lake pigments. Although lacking the fingerprint capability of vibrational spectra, it offers valuable knowledge into the paint formulation. Recipes' specificities can provide insight into chronological and location particularities, such as *scriptoria*, enabling a better understanding of the making of the artists' materials.

The ***first part*** focuses on the development and testing of modeling strategies applied to *i)* a database of historically accurate reproductions of four natural red colorants namely brazilwood, cochineal, kermes and lac dye, used during the Middle Ages; *ii)* data from artworks, to address the difficulty of analyzing centuries old paints. The first confirmed the potential for microspectrofluorimetry in the assessment of the chromophore's environment, i.e., the paint formulation, while the second explored the intricacies of the 'original' colors and the effectiveness of this methodology to explore similarities between naturally aged paints.

This project proves the ability of microspectrofluorimetry as a powerful technique for the characterization of dyes and lake pigments. The historical reconstructions database allowed to pinpoint the main recipes of cochineal lake pigments from the 19th century Winsor & Newton's database. The artworks database allowed to better understand recipe specificities and for the first time, we could pinpoint a formulation in which lac dye and brazilwood chromophores are admixed, in manuscripts from the Alcobaça *scriptorium*.

In the ***second part***, the methodology developed was tested in two case studies: the Ajuda Songbook and a group of Islamic manuscripts. The first, the oldest of the surviving Galician-Portuguese songbooks, is an unfinished illuminated manuscript, of which there is no knowledge of the circumstances of its production or the reason why it was never finished. The combination of a multi-analytical approach with the methodology developed in this project enabled the complete molecular characterization of the paint colors. It was shown the skillful construction of the paint layers and the richness of the chromatic palette, which demonstrates the desire and the resources to produce a luxurious manuscript. The methodology allowed to propose a production date for the Ajuda Songbook, in which the presence of brazilwood lake pigment and mosaic gold indicates a 14th century date, while the use of orpiment yellow pushes the date back into the 13th century.

The second case study is a group of Islamic manuscripts (12th – 15th c.), from Timbuktu, Mali, which due to their rescue and conservation have allowed the study of their materials and techniques. For the first time, the richness and specificities of the paint formulations used were disclosed. It was possible not only to provide an unequivocal molecular characterization of the red colorant, lac dye but also to ascribe

specific recipes to the different paint colors. Interestingly, the comparison with the artworks' database has shown similarities with lac dye formulations found in Portuguese medieval illuminations. Moreover, the full characterization of the paint materials has also revealed severe degradation of the binding media. This approach will allow for better informed decision-making in the conservation process of these manuscripts.

The synergy between the multi-analytical approach for the analysis of medieval manuscripts and the new methodology for the study of organic colorants was essential to the study of both case studies. The confocal spectrofluorimetry set-up used, as well as the expertise in the characterization of artworks, enabled in-depth knowledge into the construction of color paints, well beyond the identification of the single fluorophore.

Keywords: Medieval manuscripts, Dyes, Lake pigments, Spectrofluorimetry, Ajuda Songbook, Islamic manuscripts.

Symbols and Notations

λabs	Absorption wavelength
λexc	Excitation wavelength
λem	Emission wavelength

Alc	Alcobaça (normally associated with a manuscript number)
ANTT	Arquivo Nacional da Torre do Tombo
BNP	Biblioteca Nacional de Portugal (National Library of Portugal)
BPMP	Biblioteca Pública Municipal do Porto
DCR FCT NOVA	Department of Conservation and Restoration of the Faculty of Sciences and Technology, NOVA University of Lisbon
fol./fols.	folio or folios
FORS	Fiber Optics Reflectance Spectroscopy
HPLC-DAD	High Performance Liquid Chromatography coupled with a Diode Array Detector
HRMS	High Resolution Mass Spectrometry
Lv	Lorvão (normally associated with a manuscript number)
microEDXRF	Micro-Energy Dispersive X-ray Fluorescence Spectroscopy
microFTIR	Micro-Fourier Transform Infrared Spectroscopy
microRaman	Micro-Raman Spectroscopy
NIR	Near infrared
PNMAF	Palácio Nacional de Mafra
SC	Santa Cruz (normally associated with a manuscript number)
SERS	Surface-Enhanced Raman Spectroscopy
UV-Vis	Ultraviolet-visible
v	verso (the back of a folio)
W&N	Winsor and Newton

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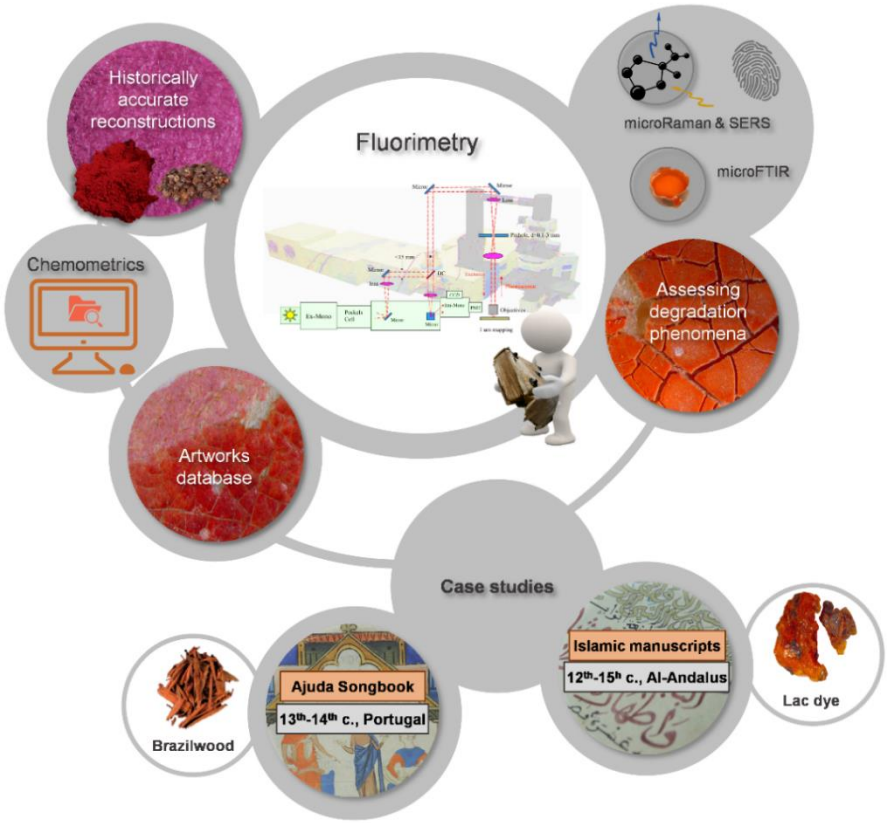
Synopsis

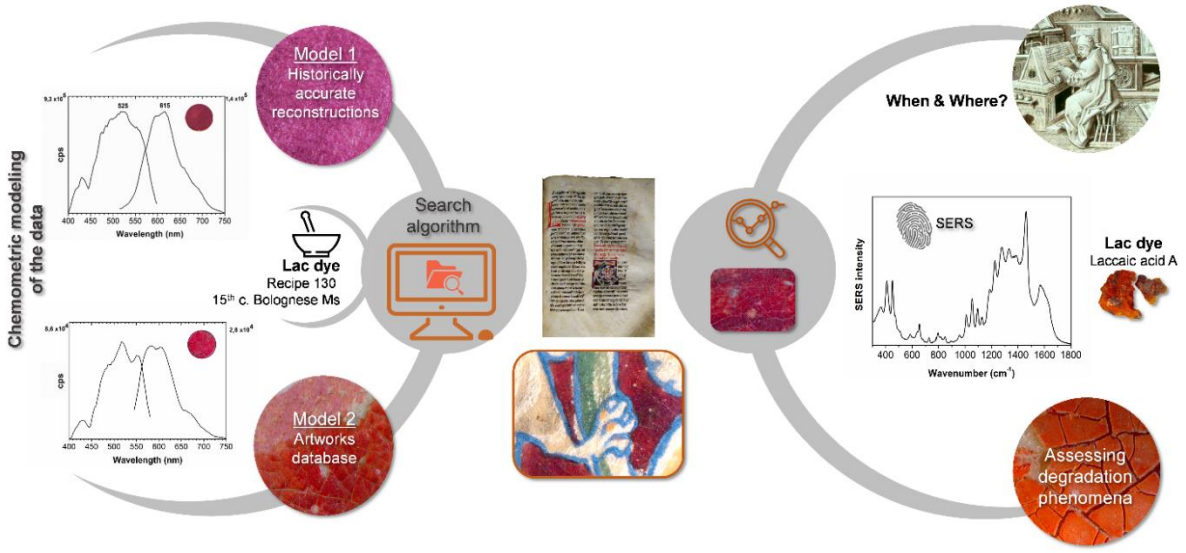
This thesis contributes to the characterization of one of the most challenging color materials found in medieval manuscript illuminations: organic colorants [1, 2]. The development of an innovative diagnostic methodology, combining fluorimetry[1] with chemometrics, allowed for the identification of the chromophore as well as the characterization of the paint formulation [1, 2]. This methodology is applied and tested in two case studies, Ajuda songbook (13th – 14th c.) and a group of Islamic manuscripts (11th – 15th c.), **Scheme 1** [3, 4]. This research was carried out within the interdisciplinary team led by Maria João Melo and Maria Adelaide Miranda, at the Department of Conservation and Restoration (DCR) at FCT NOVA. In the past decade, this team has studied illuminated manuscripts, developing an approach for the molecular characterization of the color materials, such as pigments, dyes, binders and other additives, disclosing the complexity of the paint formulation [5-8]. This detailed characterization is necessary to understand the conservation state of the medieval illuminations and advance knowledge towards their safety.



Scheme 1. Thesis methodology: fluorimetry, allied with chemometrics and robust databases, is a powerful tool for the characterization of color paints. This data is backed with fingerprint techniques which also allow the identification of degradation phenomena. This methodology was applied to two case studies, the Ajuda Songbook, and five Islamic manuscripts from Al-Andalus.

[1] The technique used in this doctoral project is denominated as fluorimetry, spectrofluorimetry or microspectrofluorimetry.

Microspectrofluorimetry presents several advantages comparing with other techniques, such as the simultaneous acquisition of emission and excitation spectra, offering high sensitivity and selectivity combined with good spatial resolution and the possibility of in-depth profiling. It can also be used in situ, without any contact with the sample or work of art to be analyzed, becoming a powerful tool for the characterization of organic colorants [1, 2, 9-11]. However, it lacks the molecular fingerprint as disclosed in vibrational spectra, which can be overcome by complementing it with micro-destructive techniques such as SERS (surface-enhanced Raman spectroscopy). While SERS provides a conclusive molecular fingerprint of the chromophore, microspectrofluorimetry offers valuable knowledge into the paint formulation [9].



Scheme 2. The approach developed in this doctoral project for the analysis of organic colorants.

Using chemometric modeling of the data acquired from the spectrofluorimeter, this doctoral project intends not only to identify the colorant present but also to address the complexity behind the color formulation, see **Scheme 2**. Recipes' specificities can provide insight into chronological and location particularities, such as *scriptoria*, enabling a better understanding of the making of the artists' materials. Therefore, the ***first part*** focuses on the development and testing of modeling strategies applied to a database of historically accurate reproductions of four natural red colorants, used during the Middle Ages: lac dye, kermes, cochineal, and brazilwood. Several modeling strategies were explored, enabling a complete discrimination of the colorants, and allowed to address the paint formulations, namely, to pinpoint the main manufacturing processes of 19th century cochineal lake pigments [12].

Following the development and application of this methodology, it was necessary to address the complexity of naturally aged color paints, such as those found in artworks, in a ***second part*** of this project. The red colorants selected for this study, lac dye and brazilwood, were identified in artworks by molecular fingerprint techniques[2]. For both these colorants, an extensive database of medieval

[2] Kermes has not been identified in artworks by the DCR's team thus far, and so, it is not included in the methodology developed for the second part of this study. Cochineal is discussed in **Appendix A1.2.**

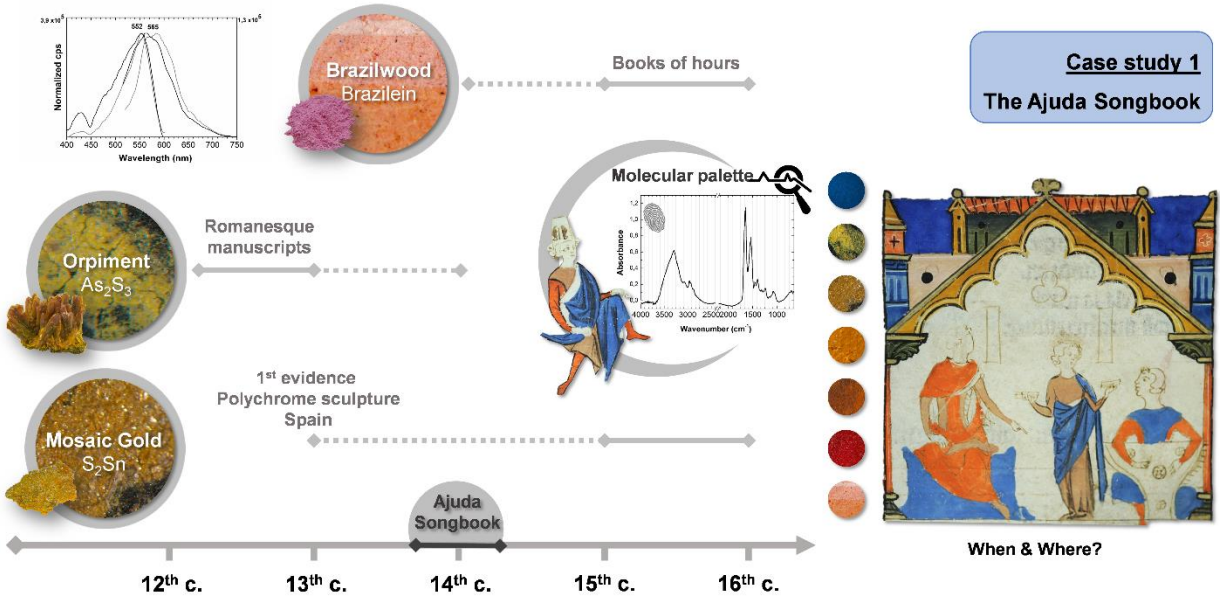
manuscripts (11^{th} – 15^{th} c.) has been investigated during the past decade by the team at DCR FCT NOVA (155 spectra) [5, 6, 11]. The modeling applied allowed for the discrimination between the colorants tested as well as a deeper understanding of the making of the colors and paint formulations present in historical artworks, enabling advances in art technological source research. For the first time, we could pinpoint a formulation in which lac dye and brazilwood chromophores are admixed, in manuscripts from the Alcobaça *scriptorium*, while probing the recipes' specificities. A ***third part***, in future development, consists of the preparation of an algorithm-based software which performs statistical modeling to generate a prediction, the colorants' identification.

The development of this methodology was tested in two case studies, integrated within a multi-analytical approach: the Ajuda Songbook, the oldest of the known existing codices of the Galician-Portuguese tradition, of which there is no knowledge of the circumstances of its production; and five Islamic manuscripts, rescued from Timbuktu in extreme conditions, which are now a challenge for conservators. The study of these two sets of manuscripts, allow this approach to be tested in very diverse typologies from different ambiences.

Case study 1. The Ajuda Songbook

The Ajuda Songbook, presently at the Ajuda Library (Biblioteca da Ajuda), an illuminated but unfinished manuscript, is a testimony of an original production of medieval Iberian cultural heritage, the Galician-Portuguese songs, commonly known as *cantigas*, see **Scheme 3**. Of this manuscript, it is not known who commissioned it, its date, the location of its production, or the reason why its text, music, and illuminations were never finished [3]. Within a collaborative and multidisciplinary approach, gathering experts from conservation science, chemistry, art history, technical art history, literary studies, and music history, this study filled in some of these gaps in scholarly knowledge about the Ajuda Songbook.

A multi-analytical approach, in which the methodology described previously was employed, allowed the full molecular characterization of the colorants present in the Ajuda Songbook, helping to clarify the questions regarding the circumstances of its production, see **Scheme 3**. The manuscript presents several colors common to the monastic palette of the 12^{th} c., including the particular and consistent use of lapis lazuli throughout the entire manuscript. It is in an impressive conservation state, which has never been encountered in previous studies of medieval manuscripts carried out by the team at DCR. The answer might be in the paint formulation and the binder used, and the pigment-binder ratio, which will be further explored. The application of the green color is also of note, where a yellow pigment is applied on top of a layer of blue or black pigment. This use of a mixture, named *vergaut*, instead of a pure green colorant allows, by the meticulous juxtaposition of the materials, for the creation of a delicate *matiz*, as well as shades and highlights [3].



Scheme 3. Multi-analytical characterization of the Ajuda Songbook (end of the 13th – beginning of the 14th century). The use of specific pigments, such as brazilwood, orpiment, and mosaic gold, together with the complete molecular palette, provide clues on the circumstances of the manuscript' production.

In the same way, it was interesting the identification of brazilwood in a manuscript produced before the discovery of Brazil by the Portuguese in 1500. However, prior to this transatlantic discovery, high amounts of Asian sapanwood, *Caesalpinia sappan*, were exported from Sri Lanka, India, and Southeast Asia to Europe during the Middle Ages [8]. According to Pastoureau, it became a popular color in fashion among the 14th century aristocracy, before being applied in books of hours of the 15th century [8, 13, 14]. Other two pigments found give essential indications on the manuscript's dating: orpiment and mosaic gold. The first was a prominent color in Romanesque manuscripts, up to the end of the 13th century. It is no longer identified in 15th c. books of hours, being replaced by non-toxic yellows, such as mosaic gold [3]. The first example of the use of mosaic gold is reported in an important polychrome statue of Santa Ana, in Santa Maria la Real, Burgos, Spain, that dates from the end of the 13th century [15]. In manuscripts, however, its identification is quite rare and was only identified in documents from the 15th century [15-17].

Concluding, if the presence of brazilwood pigment lake and mosaic gold indicates a 14th-century date for the Ajuda Songbook, the use of orpiment yellow pushes the date back into the 13th century. Nonetheless, the skillful construction of the paint layers and the richness of the chromatic palette used in the Ajuda Songbook demonstrates the desire and the resources to produce a luxurious manuscript. Further studies will be conducted, comparing it with possible *scriptoria*, such as that of Alfonso X, and other manuscripts, by studying the *Cantigas de Santa Maria*. This proposal was recently financed by the Portuguese Foundation for Science and Technology in project STEMMA[3].

[3] Project STEMMA, 'From singing to writing – survey on material production and routes of Galician-Portuguese lyric', PTDC/LLT-EGL/30984/2017; coordination: Maria da Graça Videira Lopes (PI) (IEM) and Maria João Melo (co-PI) (DCR-LAQV/Requimte, FCT NOVA).

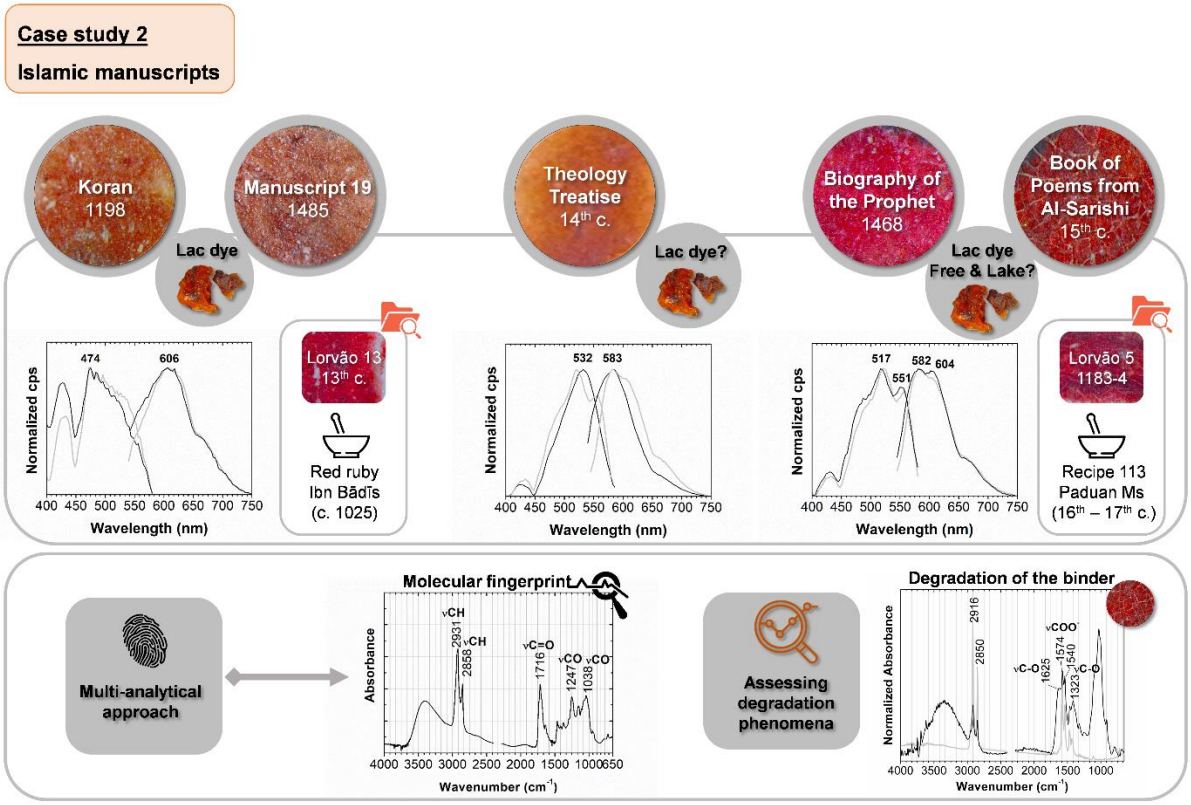
Case study 2. Islamic manuscripts from Al-Andalus

The rescue and conservation conditions of a group of Islamic manuscripts (12th-15th c.), from Timbuktu, Mali, have created the opportunity to study the materials and techniques used to illuminate these medieval books, as the second case study on this doctoral project. Until 2012, the manuscripts were stored in Timbuktu's libraries [4, 18-20], when their integrity was at risk due to the imposition of the Sharia law by a faction of the Tuareg tribe. The media and other sources describing this event, report that a librarian, Abdel Kader Haïdara, organized a plan for the rescue of more than 370 000 manuscripts, through the course of several months [18-20]. This has led to the works being hidden and transported in dramatic conditions, which significantly affected their conservation state [20].

The manuscripts chosen for this study, a Koran (1198), a Theology Treatise (14th c.), a Book of Poems from Al-Sarishi (15th c.), a Biography of the Prophet (1468) and Manuscript 19 (1485), were probably produced in the Andalusian territory, or under the Andalusian school diaspora, and are currently kept at the Timbuktu Andalusian Library (Biblioteca Andalusi de Tombuctu, *Fondo Ka'ti*). The molecular study of these five manuscripts allowed to better understand the production of Andalusian manuscripts, but also the degradation phenomena affecting these artworks. This doctoral work will focus on the organic colorants [4].

The gap in scholarly knowledge of the use of organic colorants in medieval Islamic manuscripts is vast, and to the best of the author's knowledge, in only three publications organic colorants were unequivocally identified in Islamic manuscripts; indigo was detected by micro-Raman and HPLC-DAD, while carminic acid was characterized by SERS [21-23]. The other publications rely primarily on XRF and FORS that indicate the possible colorants present. Therefore, the use of the methodology developed in this doctoral project, combined with fingerprint techniques, proved to be a powerful tool in the characterization of these artworks, identifying the main red colorants present, but also the recipes' specificities, **Scheme 4**.

Applying the methodology developed in this project, the use of lac dye in Islamic manuscripts was proven for the first time, disclosing the richness and specificities of the formulations: all lac dye based, but all different. With the exception of the Theology treatise, for which it was not possible to provide an unequivocal molecular characterization of the main chromophore, the approach ascribed a specific recipe for the production of the colorant and disclosed the paint formulations in the several manuscripts. It was possible to further match the colorants with recipes from the historically accurate reproductions database (recipes from the Ibn Bādīs and the Paduan manuscripts) and with data from Portuguese manuscript illuminations (Lorvão 13 and Lorvão 5, from the Monastery of Santa Maria of Lorvão). Moreover, applying a multi-analytical approach, the spectra matched with fluorimetry spectra of the database, as well as with infrared data, which allowed the identification of the resin from the lac dye and binders used. It also enabled to conclude that the chromophore is well preserved, but the binding media show signs of severe degradation. To further assign the use and production of lac dye in Islamic manuscripts, other case studies which were produced in Al-Andalus will be studied in the future.



Scheme 4. Multi-analytical characterization of the organic reds found in the Islamic manuscripts. It was possible to identify for the first time, lac dye in Islamic manuscripts. The approach allowed to further ascribe the signals to specific recipes and compare with lac dye applied in Portuguese manuscripts.

The confocal spectrofluorimetry set-up available, as well as the expertise in the characterization of artworks, gathering a multi-analytical approach and a robust database of historically accurate reconstructions developed by the team at DCR FCT NOVA, have placed this doctoral work in a privileged position to test the efficacy of multivariate data analysis on fluorimetric measurements. This approach has, for the first time, provided higher insight on the construction of color paints, well beyond the identification of the single fluorophore. It can disclose important information on recipes' specificities, leading to chronological and location particularities, such as *scriptoria* and artists' workshops, enabling a better understanding of the making of the artists' materials. This innovative methodology, combining fluorimetry with chemometrics, allowed for the identification of brazilwood in the Ajuda Songbook, and lac dye, both as colorant and a a lake pigment, in the Islamic manuscripts. The characterization of these colorants revealed their conservation condition, but also the richness and diversity of the paint formulations used. Moreover, the combination of the methodology developed in this doctoral project with the multi-analytical approach of the team at DCR, proved very useful in the approach to the two case studies. For the analysis of cultural heritage, no technique alone can solve the complex problems encountered, whereas the synergistic combination of several techniques allows for in-depth characterization of artists' materials, required for a well-designed preservation or conservation intervention.

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This thesis is based on the following publications:

Part 1. Microspectrofluorimetry for the safeguard of dyes and lake pigments

Chapter 2. Microspectrofluorimetry and chemometrics for the identification of medieval lake pigments

Paula Nabais, Maria João Melo, João A. Lopes, Tatiana Vitorino, Artur Neves and Rita Castro, "Microspectrofluorimetry and chemometrics for the identification of medieval lake pigments", Herit Sci. 6:13, 2018.

Chapter 3. Organic colorants as makers for a chronology and geography of medieval scriptoria and workshops: a chemometrics approach

Paula Nabais, Maria João Melo, João A. Lopes, Márcia Vieira, Rita Castro and Aldo Romani, "Organic colorants as makers for a chronology and geography of medieval *scriptoria* and workshops: a chemometrics approach", *Forthcoming.*

Part 2. Case studies[4]

Chapter 4. Singing with light: an interdisciplinary study on the medieval Ajuda Songbook

Paula Nabais, Maria João Melo, Rita Castro, Luís Sousa and Graça Videira Lopes, “Singing with light: an interdisciplinary study on the medieval Ajuda Songbook”, Journal of Medieval Iberian studies, Routledge: Taylor & Francis, theme issue Sci-ArtH: Interdisciplinary Approaches to Medieval Iberian Heritage, 2016, pp. 283-312.

Chapter 5. Organic reds in Islamic manuscripts (12th-15th c.) produced in al-Andalus

Márcia Vieira, Paula Nabais, Eva Mariasole Angelin, Rita Araújo, João Almeida Lopes, Lourdes Martín and Marta Sameño, "Organic reds in Islamic manuscripts (12th-15th c.) produced in al-Andalus, part 1", Dyes & Pigm., 2019;166:451-9.

[4] Both case studies were analyzed by a team. For the Ajuda Songbook the team responsible for the analysis of materials was composed of Maria João Melo, Paula Nabais and Rita Castro. For the Islamic manuscripts the team was Maria João Melo, Paula Nabais, Márcia Vieira, Ana Luís, Rita Araújo and Eva Angelin. For both case studies, the auhor of this doctoral dissertation participated in the acquisition and discussion of the data and the writing of the articles.

General Introduction

Detail from the Ajuda Songbook, 13th – 14th centuries: Musician, fol. 21 © Ajuda Library

Chapter 1. General introduction

The characterization and identification of organic dyes is still a main challenge for Conservation Science, to which this thesis plans to contribute. By exploring the potentialities of microspectrofluorimetry with chemometrics, this work intends to provide not only an identification of the chromophore but to get in-depth knowledge about the making of these colors. Information about recipes' specificities, will allow us, in the near future, to propose chronologies for the use of organic colorants, and possibly even appointing specific *scriptoria* which used and prepared these colors, and their geographical area of influence. This thesis is focused on the red colorants, widely used in artworks, from textiles to medieval illuminations and 19th century paintings. The extensive work carried out by the group led by Maria João Melo at the DCR FCT NOVA, over the last ten years, on the historically accurate reconstructions of red lake pigments has provided a robust database, suitable to develop the approach explored in this thesis. This is presented in **Chapter 1** of this thesis. In **Chapter 2** this methodology is tested on data collected from artworks. In **Chapters 3** and **4**, this approach is then applied on two case studies, where the red organic colorants applied are identified, and their paint formulations disclosed[5]. This work will contribute to the future development of an algorithm-based software which performs statistical modeling to generate a prediction, which will enable the colorant identification and other recipe' specificities.

1.1. Fluorimetry for the characterization of organic colorants in artworks

The characterization of natural organic colorants[6] in artworks in still a challenge to this day. The difficulty of analyzing these materials is sometimes allied to frequent impossibility of microsampling, and the frailty of the artworks. One of the main techniques used for the characterization of these materials is high-performance liquid chromatography (HPLC), which coupled with diode-array detection and mass spectrometry is a very robust technique for the identification of organic colorants [1-6]. It allows the characterization of all chromophores present, and new compounds (with mass spectrometry), enabling the identification of the source material. Although being extensively used for the characterization of textile materials, HPLC still required a large sample, up until the very recent development of ultra-high-performance liquid chromatography (UHPLC), which has improved in sensibility with a smaller sample size [6]. However, in some artworks, namely medieval illuminations, the frailty of the paint layers prevents us from removing samples large enough for this analysis. Raman microscopy is another technique commonly used for the characterization of organic colorants. The masking of the chromophores' signal with this technique, due to the intense fluorescence, can be overcome by surface-enhanced Raman spectroscopy (SERS) and other methods such as subtraction shifted Raman spectroscopy (SSRS) [7]. Although SERS requires a micro-sample it provides fingerprint identification of the chromophore present [8-9]. On the other hand, SSRS requires spectral conversion, which

[5] The purple dyes identified in both case studies are described in the **Appendix 2**.

[6] Generally, colorants derive from both mineral (inorganic) and animal or plant-based (organic). A pigment is a substance which is non-soluble in the vehicle, e.g., water or binder, while a dye is a water-soluble substance.

generate erroneous bands, requiring the repetition of independent sets of experiments to exclude these features [7].

Over the past decade, the development of analytical techniques and new approaches have proved useful to overcome the difficulty in analyzing all paint components. Fiber optic reflectance spectroscopy (FORS), is proving to be a promising tool for pigment, but also dyes and binder identification. This technique may have access not only to the visible range (380 – 700 nm) which enables the identification of some colorants, namely pigments, but also to the UV region (200 – 380 nm) as well as the near-infrared (NIR) range (750 – 1100 nm) which adds specific features useful for the identification of these materials [10]. Reflectance hyperspectral imaging has been developed recently, allowing the identification and spatial distribution of colorants, binders, and additives, by the collection of continuous calibrated spectral images which provide reflectance spectra for each pixel of the image [11-14]. On the other hand, the complementarity use of specific techniques has proven to be a robust methodology. Surface-enhanced Raman spectroscopy (SERS) and microspectrofluorimetry in the visible (Vis) have proven to be powerful techniques for the identification of dye-based paints [15-20]. The first, requires microsamples as small as 25µm, allowing for significant enhancements of the Raman scattering for substances which are adsorbed on metallic nanoparticles, proving to be an invaluable tool in the identification of fluorescent molecules [8, 9]. The second, microspectrofluorimetry, allies high sensitivity and selectivity with the possibility of in situ analysis [15-17].

1.1.1. Natural organic colorants in artworks from Antiquity until the 19th c.

Red and purple dyes give such intense and bright colors that have long been used in the textile industry, as well as by illuminators and painters [15, 21, 22]. Because of their importance in the commercial routes of the Middle Ages as well as in the production of many artworks, they are considered part of the worlds' cultural heritage, having received attention by conservation scientists and art historians [22]. Some natural dyes were used for millennia, up until the advances in modern chemistry, which allowed for the development of synthetic dyes. The red and purple colorants described below belong to a chronological range from the beginning of the Mediterranean Classical Civilizations until the 19th century[7] that have been studied by authoritative experts on natural organic colorants, such as Dominique Cardon and Jo Kirby, Maarten van Bommel & André Verhecken[8] [21, 22]. See **Figure 1.1** for the structures of the most relevant dye classes.

The primary sources for red natural organic colorants, either identified in artworks or described in medieval treatises and recipe books, were madder, lac dye, cochineal, kermes, and brazilwood. All, excepting the last, are composed by anthraquinones hydroxy derivatives. [21, 22].

[7] The number of organic dyes used within the period selected is vast, and depended on geographical location, i.e. Europe, Americas, Asia. Therefore, this work focused on the main sources of dyes in Europe, as described in literature [21, 22].

[8] One of the most important European projects on the study of natural organic colorants was the CHARISMA (Cultural Heritage Advanced Research Infrastructures – Synergy for a Multidisciplinary Approach to Conservation/Restoration), a Research Infrastructure funded by the European Union 7th Framework Programme (2009-2014, Grant Agreement no. 228330).

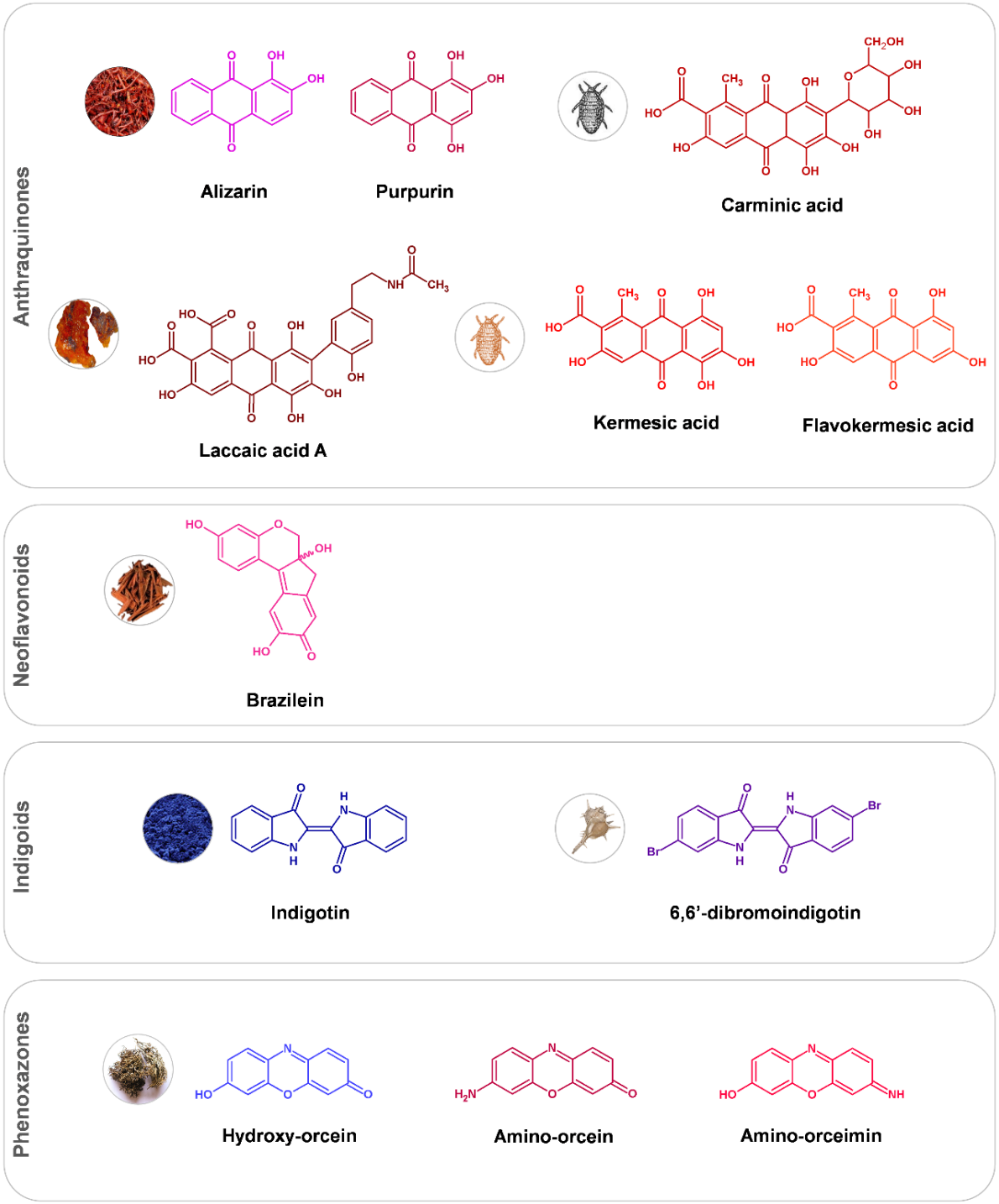

Figure 1.1. Structures of the most relevant dye classes and compounds found in blue, purple and red natural organic colorants.

Dyer's madder is obtained from the plant *Rubia* spp., namely the *Rubia tinctorum* L., and has been one of the most used dyestuffs since Ancient Egypt [19, 21, 23, 24]. It is mentioned in treatises as early as that of Pliny, *Historia Naturalis*, which describes the use of the roots to extract the dye [23]. The *Rubicae* family includes several madder plants, although the *Rubia tinctorum* L. is the most known. Other madders included Indian madder (*Rubia cordifolia*) and wild madder (*Rubia peregrina*) [21, 24]. Large-

scale cultivation of madder occurred in Europe since the Middle Ages which carefully took into account factors such as cultivation, climate, and soil to achieve the best quality dyestuff [23].The highly prized Turkey red, the method of madder dyeing on cotton material, introduced in Europe in 1746, became an industry on its own and by 1896 the dyeing businesses joined to form the United Turkey Red Company, which rapidly declined after World War I, closing in 1939 [21, 23]. Much like other dyestuffs, madder was also subject to forgeries with brick powder, sand and dyed sawdust [23]. The chromophores present are alizarin, the main colorant for the majority of the madder species, and purpurin, a highly fluorescent molecule.

Lac dye is a resinous secretion produced by the insect *Kerria lacca*. It was extensively used from pre-historic times to the Middle Ages, being widely found in manuscripts from *scriptoria* of the main Portuguese Monasteries [18, 20]. Lac scale insects were found in southern and southeastern Asia, namely India, in various trees, such as those of the *Ficus* family (*Ficus indica*, *Ficus religiosa*) forming colonies surrounded by an encrustation of the resinous lac [20, 21, 23]. Several months after the fertilization of the female in January, the secretion becomes thick, sometimes up to a few centimeters. It is at this stage that the harvest of lac dye red would begin, where the twigs of the trees were removed and placed in the sun to dry and kill the insects [23]. The dyestuff would then appear as a yellowish red to dark brown resin, called sticklac [24]. This resinous material was then processed, separating the dyestuff from the resin, named shellac. The deep red color is given by the presence of laccaic acids A, B, C, D, and E, of which the first is the main chromophore. The best quality lac was exported to other countries from the earliest center of production, Indochina [24]. For more information, see **Appendix 1**.

Kermes is a dye which, similarly to cochineal, is extracted from an insect, *Kermes vermilio*, a parasite found in the kermes oak (*Quercus coccifera* L.). Kermes was the red dye *per excellence*, the symbol of richness and power, with its apogee in the Middle Ages after the downfall of Tyrian purple, being known in Antiquity by *coccum*, *coccus* or *granum* [21, 23]. In fact, it was in 1467 that Pope Paul II officially replaced purple by this scarlet red, becoming a very expensive dyestuff [23]. Much like cochineal, the chromophores, kermesic acid and flavokermesic acid, are present in the unlaid eggs of the female insect [21, 24]. The collection of the pregnant female insects occurred before the egg laying in May and for one gram of dyestuff, 60 to 80 insects were needed [21, 23]. The harvest of the insects was a delicate a laborious job, detaching each insect with long fingernails. By the middle of the 17th century, kermes had been replaced by cochineal [24].

Cochineal dye is extracted from the insects *Porphyrophora polonica* (Polish cochineal), *Porphyrophora hamelii* (Armenian cochineal) and from the *Dactylopius* genus, such as *Dactylopius coccus*. The first two were widely used in Europe during the Medieval Ages, up until the discovery of the New World, which led to the importation of the *Dactylopius* genus, used by the Aztec, Maya and Incan civilizations [21, 24]. The pre-Columbian civilizations used this insect for dyeing textiles and illuminating manuscripts since the 11th century and the Náhuatl (old Aztec) term for the color produced was *nocheztli*, later changed to *cochinilla* by the Spanish [23, 24]. Despite being introduced in Europe in the 16th century, the existence and production of cochineal red was kept a secret by the Spanish until the 18th century [23]. The colorant is extracted from the eggs of the pregnant female insects, and the main chromophore

is carminic acid [19, 21, 22, 24, 25]. The American cochineal insects are found in the *Opuntia* spp. cacti, introduced to Europe before 1550 and are collected in two to four harvest seasons [21, 23, 24]. The insects were collected carefully and then killed by different methods, one of which consisted in placing them in the heat of the sun in the warm sand; this was considered to yield the best product, the silver cochineal, *blanca* or *plateada* [21, 23]. The large-scale production of this dyestuff in the Americas led to the implement of large plantations with no less than 50,000 cacti, becoming one of the main sources of colonial income, and the Spaniards held the monopoly until the 1700s [23].

Brazilwood was imported to Europe, during the early Middle Ages, from Sri Lanka, India, and Southeast Asia, and later from Brazil after the discovery of this country by the Portuguese in the 16th century [24, 26]. Brazilwood was named *brazil*, *brasil*, *bresil* or *brasilium*, due to fiery red color of this dye (*brasa* means glowing coals in Portuguese) and it achieved such an importance in colonial economy that led to the change of name of the country known as *Terra da Vera Cruz* (Island of the True Cross) to Brazil [21]. It became widely used as a lake pigment for paintings and manuscripts, having been found in French books of hours present in Portuguese collections [26, 27]. When imported from Asia, the species used was *Caesalpinia sappan*, which began to be gradually replaced by those imported to Europe from South America, namely *Caesalpinia echinata*, *Caesalpinia brasiliensis*, among others [26, 27]. The main compound present is the almost colorless flavonoid brazilin which oxidizes to brazilein, the chromophore responsible for the red color [26, 27].

The blues were mainly produced by indigo, which is an organic colorant obtained, in Europe, from woad (*Isatis tinctoria L.*) and *Indigofera* spp. The molecule responsible for the intense dark blue color is indigotin [22, 28]. Indigo was the blue used worlwide in the textile industry, but also in paintings and manuscripts, and still had major importance in the 21st century [28]. From the same family of chromophores, a purple color monopolized for centuries the dyeing industry. Tyrian purple was extracted from the gland of sea mollusks, such as the *Murex* spp. [21, 23]. Because a mollusk gland gives only minimal quantities of dye, millions were needed to accommodate the royal requests of dyed works. The name is given from a specific production site, the powerful Phoenician city of Tyre and purple from the ancient Greek word *porphura* [23]. The primary color component is 6,6'-dibromoindigo [21]. Due to the high prices and production cost of Tyrian purple, as well as to the scarcity of mollusks in specific regions due to extensive harvesting, other sources of purple were explored [21]. Although red colorants such as cochineal and lac dye could provide a deep purple hue, or in mixtures with indigo, other colorants were also used as substitutes for Tyrian purple: orchil and *folium*.

Orchil was produced from species of lichens, such as the *Rocella* spp., found all over the world, from mountains to the coasts of subtropical or tropical regions in Europe, Asia, Africa or South America [21]. Although less lightfast than shellfish purple, it continued to be used long after the decline of the latter [21]. At some point, the name orchil was given to a dye produced by any lichen species in ammonia [21]. After extensive use during the Classical period and the early Middle Ages, its use and production were lost and forgotten. During the Crusades, around the 13th c., Frederigo Rocellai returned from the Levant to Italy with the formula for dyeing with orchil, and due to the novelty of purple dyeing, the Florentine family was soon made famous. This fame and fortune were such that in 1763, Linnaeus

named a lichen species in their honor, *Rocella tinctoria* [21]. The main chromophores are phenoxazones, namely amino-orceins, hydroxy-orceins and amino-orceimines [29].

Another purple dye used by manuscript illuminators in the Middle Ages was known simply by *folium*. It was extracted from the fruits of the plant *Chrozophora tinctoria* (L.) A. Juss. or *Croton tinctorium* L., and herbalists have, over the centuries, variously titled it as *solsequium*, *tournesol* (or *turnsole*), *morella* (*maurelle*), *Heliotropium tricoccum* and *Croton tinctorium*. causing confusion with other plant species [21, 30]. Its main chromophore is yet to be characterized. The names given to these colorants would vary quite substantially, depending on the time and place, and the confusion with terminology has led to several names being used to define the same source of dye, or the same denomination be applied to several sources. Despite this, it was still extensively used as a dyestuff in Gallargues, a village near Montpellier, to be exported to Germany and Holland to give color to wines, liquors and confitures until the 18th century [30].

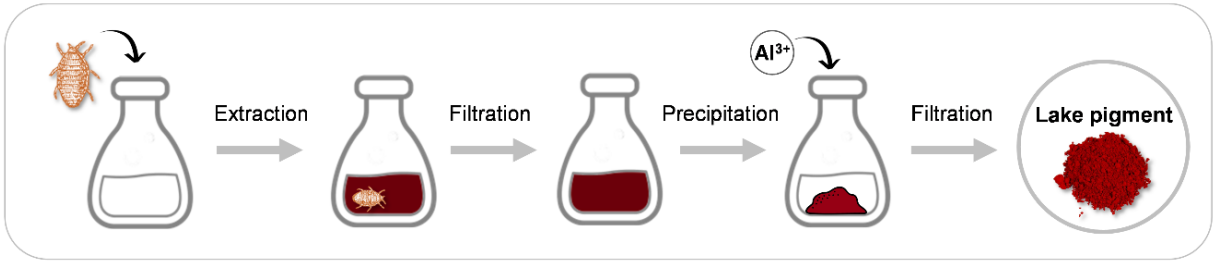

Figure 1.2. Scheme of the main steps for the reproduction of lake pigments. Image adapted from a design by Rita Carvalho and Rita Castro [18, 20].

Dyes are water-soluble substances, mostly of organic origin, which were used in three ways: *i*) as a direct dye, which binds directly to the textile, such as orchil; *ii*) complexed with a metal ion, a mordant[9], which forms a bridge between the textile fiber and the dye; *iii*) as a vat dye, which consists on the conversion of a dye which is not soluble, into a water-soluble form. Dye-mordant complexes are commonly mentioned in medieval treatises, especially for red and yellow dyes, while the mechanism described for vat dyes is typical for indigo and Tyrian purple [22]. Generally, most dyes were applied as lake pigments, formed by the precipitation of the colorant with a complexing agent, such as alum, hence becoming a non-soluble pigment, in a process analogous to the mordanting of textiles, **Figure 1.2** [22]. Other colorants, such as *folium*, were used has 'clothlets' [31]. Clothlets[10] were a way to preserve the colorants: "*any color of direct dye extracted from a flower or a berry that is stored by being absorbed into a cloth, to be subsequently released by immersion in a medium*" and applied as a watercolor [31, 32]. Although mentioning the use of *folium* as a clothlet, De Arte Iluminandi refers that the '*pezzuol*e' were not always with *folium*, but other dyes as well (chapters X and CLXI) [33].

[9] The most common mordant in the Middle Ages is aluminum in the form of alum, $AlK(SO_4)_2 \cdot 12H_2O$. Other metals could be used, such as iron, tin (from the 17th c. onwards) and chromium (from the 19th c. onwards) [22].
[10] *Pezzuole* and *pezette* were the Italian names for clothlets [31].

1.1.2. Fluorescence emission spectroscopy, a promising technique

Natural colorants, as organic compounds, are capable of emitting photons from an electronically excited state. This emission of light is called luminescence. The classes of organic compounds known to be capable of luminescence, namely anthracenes, benzopyrans, indoles and oxazines, include the compounds mentioned previously, anthraquinones, neoflavonoids, indigoids and phenoxazones, respectively, see **Figure 1.1** and **Figure 1.3**. The way a molecule arrives at the excited state depends on the phenomenon observed, and for the organic classes studied, the absorption of light is the cause of the de-excitation phenomenon named photoluminescence (fluorescence, phosphorescence and delayed fluorescence) [34].

Fluorescence, as a phenomenon, has been known since the end of the medieval period. The first published observation of fluorescence was the emission of light by an infusion of wood *Lignum nephriticum*, by Nicolás Monardes in 1565. Monardes was a medical practitioner who studied the new plant species, brought from the New World by the Spanish [34, 35]. In his book, *Historia Medicinal*, Monardes engaged on the first description of the medicinal uses of dozens of American plant species, in which the fluorescence qualities of this wood infusion were explained [35]. Parallel to this discovery, a Franciscan missionary, Bernardino de Sahagún, wrote an illustrated manuscript entitled *Historia General de las Cosas de Nueva España* (ca. 1575-1577), in which a plant named *coatli* by the Nahuatl people, is used as "*a medicine and makes the water of blue colo*r." This *coatli* was the source of *Lignum nephriticumi* [35]. Around the 17th century, other scholars pursued the complex issue of the fascinating colors of the *coatli*, namely Athanasius Kircher (1601-1680), Robert Boyle (1627-1691) and Isaac Newton (1643-1727). Important advances were made, such as the study of the infusion's sensitivity to acidic and alkaline conditions as well as the optical characteristics, as described by Newton in his influential treatise *Optiks* and on a letter to the Royal Society*,* "*those are substances apt to reflect one sort of light and transmit another*" [36, 37]. The famous astronomer John Herschel (1792-1871) also studied this phenomenon and named it '*epipolic dispersion,'* [34, 35]. It was only in 1982 that the two major compounds of *coatli* were discovered, Coatline A and B, both chalcones, a class of compounds categorized as minor flavonoids [35].

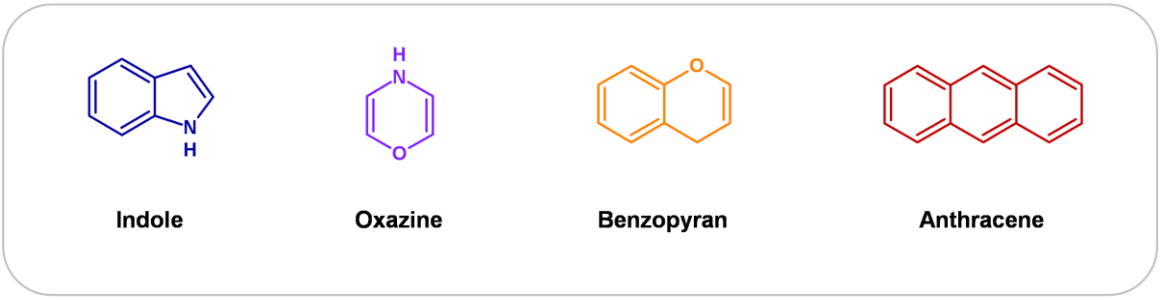

Figure 1.3. Classes of luminescent organic compounds found in the natural colorants mentioned.

Between the 16th and the 19th century, the emission of light was reported and highly addressed, namely with chlorophyll and quinine sulfate solutions [34, 38]. In 1852, George Gabriel Stokes reinvestigated the phenomena described by Herschel, publishing a famous paper entitled '*On the change of refrangibility of light*' [34, 39], see **Figure 1.4**. In this paper, he describes the sensitivity of certain natural materials, such as those of orceins, that he calls '*archil and litmus,'* in an alkaline or alcoholic solution [34, 39]. The phenomena of color produced by natural colorants, *absorption*, was already known at that period. Stokes mentioned: "*It is now admitted that the various colors of natural bodies are merely particular instances of one general phenomenon, namely, that of absorption. Absorption is most conveniently studied in a clear fluid or solid, but it does not the less exist in a body of irregular structure, such as a dyed cloth or a colored powder*" [39]. Stokes also described thoroughly the phenomena of emission of light and noted that the emitted light, that he named '*dispersive reflexion'*, was always of a longer wavelength than the exciting light: "*the incident light belongs to the visible part of the spectrum, the dispersed rays (if any), being of lower refrangibility than the incident light*" [34, 39]. This statement would later be named as the Stokes' law. In a footnote of his article, Stokes mentions his dislike for the term '*dispersive reflexion'*, stating "*I am almost inclined to coin a word and call the appearance of* ***fluorescence****, from fluor-spar, as the analogous term opalescence is derived from the name of a mineral*" [39]. In his second paper, in 1853, Stokes decides to name the phenomena of emission of light as '*fluorescence*' [40].[11] Stokes also appears to have been the first to suggest the close relation of fluorescence with the molecular structure: "*there remain three phenomena in which bodies are related to light in a manner varying from (…), but in a way depending on something in the molecular constitution of the particular body in question, (…) These are (1) absorption, (2) fluorescence, (3) colored reflexion*" [41].

During the 20th century, significant developments in the history of fluorescence occurred, allowing the use of this phenomenon for the characterization of organic compounds. In 1904, Nichols and Merritt acquired the first fluorescence excitation spectrum of a dye [34, 42]. By using a spectrophotometer, they placed a solution of fluorescein in a rectangular cell and obtained different spectral intensities by exciting in different regions [42]. They later presented spectrophotometric measurements of several dyes, such as eosin, rhodamine, and resorufin (an amino-orcein) [43, 44]. Another remarkable achievement was done by Aleksander Jabloński, in 1935, by the development of the Jabloński diagram, which illustrates the electronic states of a molecule and the transitions between them, describing the absorption and emission of light [34], see **Figure 1.4** and **Figure 1.5**. Since then, scientists have studied and addressed fluorescence spectroscopy as a technique capable of characterizing organic molecules [34].

By absorption of a photon, a molecule is excited, and returns to the ground state by several pathways of de-excitation, such as fluorescence[12], see **Figure 1.5**. The fluorescence spectrum is affected by processes that occur in the excited-state, which involve interactions of the molecule with its close

[11] "*In my former paper I suggested the term fluorescence, to denote the general appearance of a solution of sulphate of quinine and similar media., I have been encouraged to give this expression a wider signification, and henceforth, instead of true infernal dispersion, I intend to use the term fluorescence, which is a single word not implying the adoption of any theory*" [40].

[12] Luminescence is also presented in the form of phosphorescence. This is the radiative de-excitation from a triplet state, and because a transition from such a state to the ground state is forbidden, other non-radiative de-excitation processes are favored.

environment, providing crucial information on such a matter. The environmental parameters which affect fluorescence properties are the pH, temperature, viscosity, ions, polarity, hydrogen bonds, quenchers, and other factors [34]. As stated by Valeur, "*the success of fluorescence as an investigative tool in studying the structure and dynamics of matter or living systems arises from the high sensitivity of fluorometric techniques, the specificity of fluorescence characteristics due to the microenvironment of the emitting molecule, and the ability of the latter to provide spatial and temporal information*" [34].

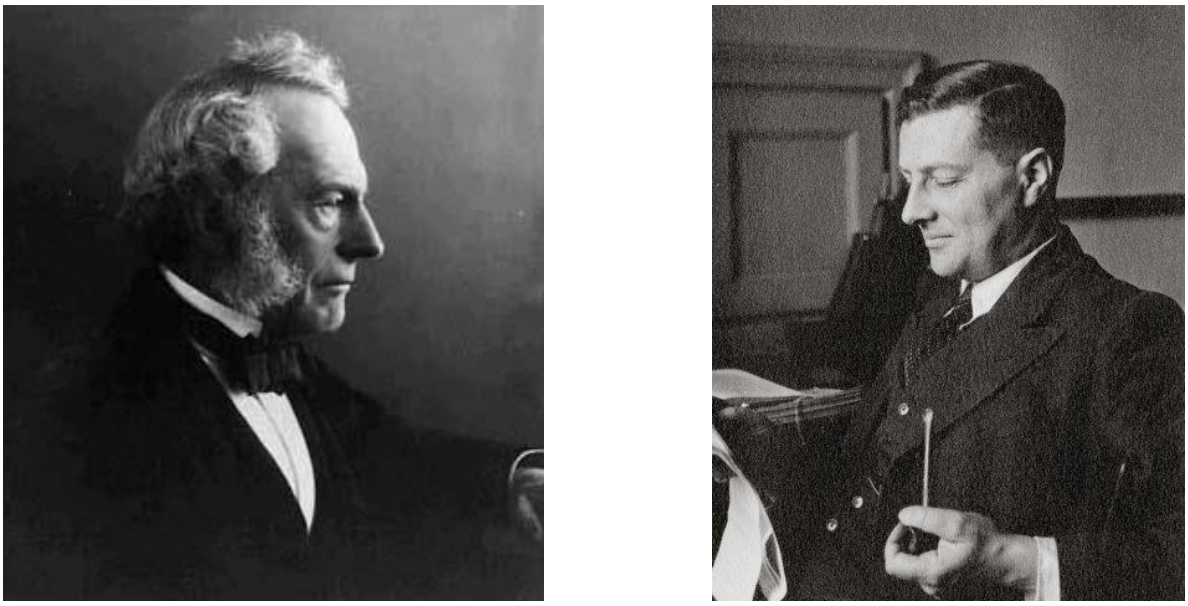

Figure 1.4. George Stokes (*left*) and Aleksander Jabloński (*right*). © School of Mathematics and Statistics and Fundacja Aleksandra Jabłońskiego.

Due to this strong influence of the microenvironment on fluorescence emission, these luminescent molecules are currently used as probes to characterize their environment, in physicochemical, biochemical and biological systems.

Analytical techniques based on fluorescence emission detection are very popular due to their high sensitivity and selectivity, together with the advantages of good spatial and temporal resolution [34]. Good spatial resolution can be obtained with the use of a confocal microscope which allows the detection of fluorescence emission even at high dilution (≈10^{-9} M or less). Good temporal resolution can be obtained using high repetition rate picosecond lasers and microchannel plate photomultipliers, providing valuable information on the lifetime of excited-state species [34].

Fluorescence emission detection is possible by means of a spectrofluorimeter operating at an appropriate excitation and observation wavelengths. A fluorescence spectrum is the distribution of the probability of the various transitions from the lowest vibrational level of S_1 to the various vibrational levels of S_0, i.e., the emission, measured at a constant wavelength, see **Figure 1.5**. This fluorescence, or emission spectrum, is characteristic of a given compound. This agrees with the Kasha's rule which states that photon emission (fluorescence or phosphorescence) occurs in appreciable yield only from

the lowest excited state. The excitation spectrum reflects the variations in fluorescence intensity as a function of the excitation wavelength for a fixed observation wavelength. This excitation spectrum should be identical to the absorbance spectrum if there is a single species in the ground state, and if the excitation spectra is corrected for the instrument response [34]. However, if several species are present or exist in different forms in the ground state, both the spectra are no longer similar. For further information on the electronic transitions which may occur in polyatomic molecules with the absorption of a photon, see [34].

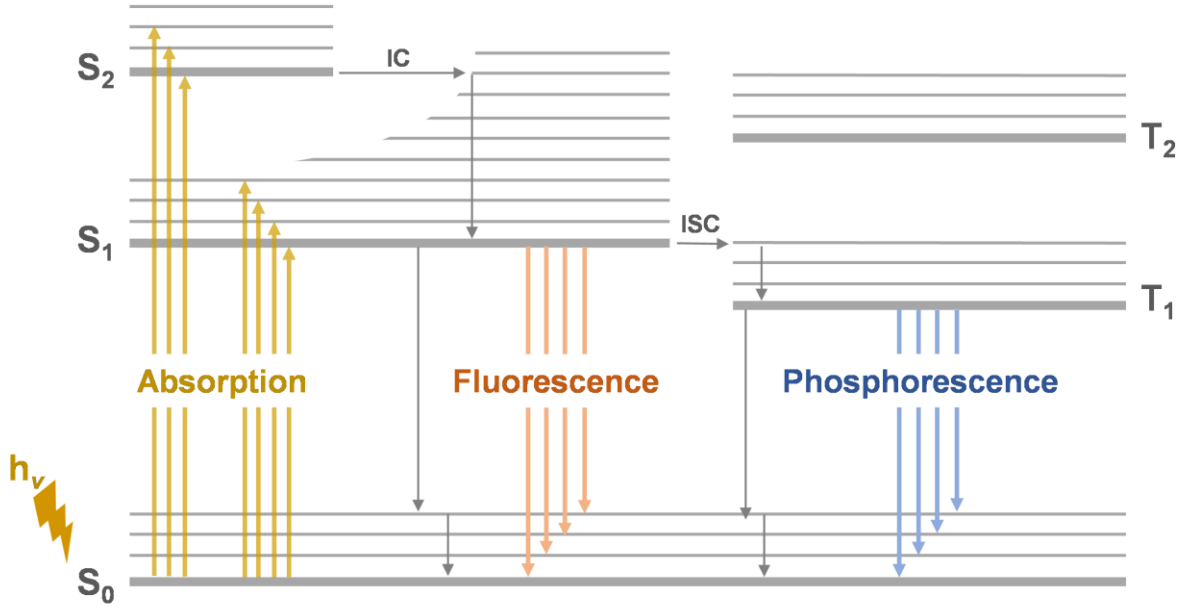

Figure 1.5. Perrin-Jabloński diagram. A photon is absorbed, and an electronic transition occurs, promoting an electron from the ground state, S_0, to other fundamental electronic states, S_1, S_2, etc. De-excitation may occur by fluorescence, phosphorescence (involving triplet states, T_1, T_2, etc.) and non-radiative processes such as internal conversion (IC) or intersystem crossing (ISC).

Before the development of the first spectrofluorimeters in the 1950s, each scientist had to either build their own or modify existing instruments to carry out fluorescence measurement studies [45, 46]. Due to the uniqueness of each equipment, the comparison of data was problematic. During World War II, the United States government urged scientists to find a treatment for malaria, which became an increasingly bigger problem in the US troops serving in remote islands of the South Pacific. Finding antimalarial drugs was urgent, since the traditional medicine, quinine, was controlled by the Japanese. Led by James Shannon in the Goldwater Memorial Hospital in New York, scientists Sidney Udenfriend and Bernard Brodie developed analytical tools to monitor antimalarial drugs being tested in patients and volunteers [46]. One of these analytical instruments was the Coleman fluorimeter, the first commercial instrument available to measure fluorescence.

The to limitations of the Coleman instrument, such as a glass-coated mercury lamp for excitation and all-glass optics (not suitable for UV), led to the development of an ultraviolet and visible spectrofluorimeter by Udenfriend and Robert Bowman, at the end of the 1950s. They faced some

obstacles, namely to obtain the supplies and instruments needed at the time, which were quite expensive, and to find a suitable light source for excitation [46]. The available lamps at that time either had insufficient energy (tungsten) or were limited to some regions of the spectrum (mercury-vapor, present in the Coleman instrument). However, during World War II, Germany developed xenon-arc lamps for their searchlights and were still available at the end of the 1950s for movie projectors. Xenon-arc lamps offer the advantage of continuous emission from ~250 nm to the infrared, and so were suitable for Udenfriend and Bowman's work [34]. At the same time, photomultiplier tubes became commercially available, and were appropriate for light detection of very weak signals, thus offering high sensitivity. The American Instrument Company (AMINCO) became interested in marketing the new instrument and collaborated on a commercial version. This was called the AMINCO-BOWMAN spectrofluorimeter and was first exhibited at the 1956 Pittsburg Analytical Instrument Conference, see **Figure 1.6** [46].

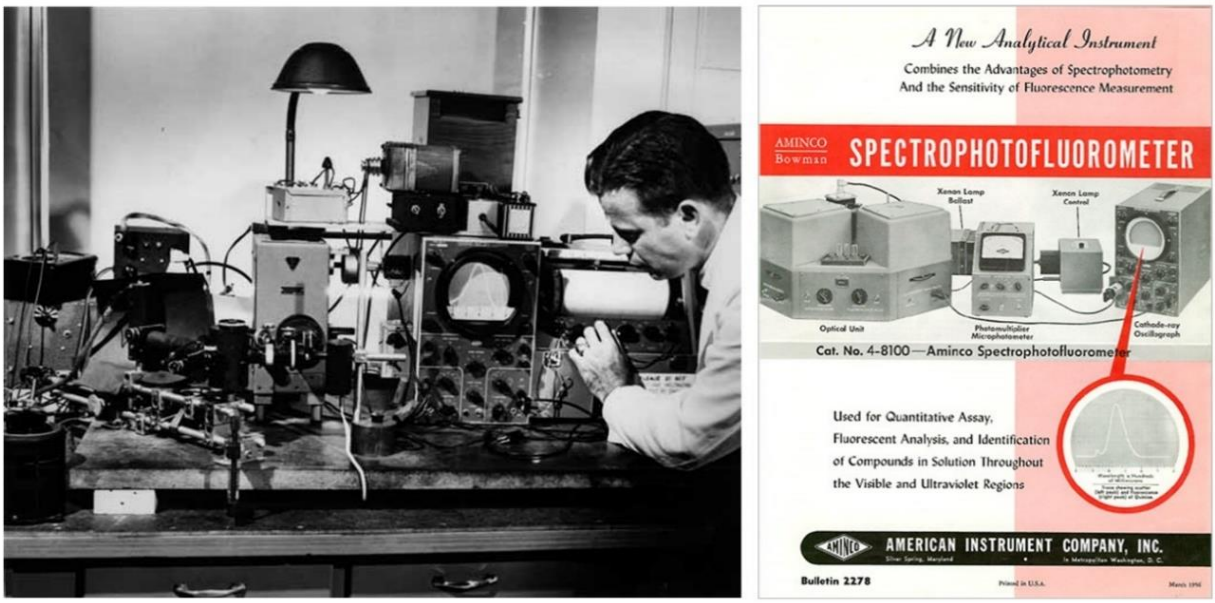

Figure 1.6. Bowman's bench-top prototype (*left*) and the first commercialized spectrofluorimeter, the AMINCO-BOWMAN (*right*), © NIH Historical Office.

Nowadays, a conventional spectrofluorimeter usually uses a high-pressure xenon arc lamp, and a monochromator to select the excitation wavelength [34]. Fluorescence is collected at right angles concerning the incident beam and detected through a monochromator by a photomultiplier. Automatic scanning of wavelengths is achieved by the motorized monochromators, which are controlled by the electronic devices and the computer. The samples must be diluted enough to avoid some undesirable effects such as self-absorption [34, 45].

1.1.3. Fluorescence spectroscopy for the analysis of dyes in artworks

The use of fluorescence techniques also became of interest to scientists working in the field of cultural heritage, and the development of the spectrofluorimeter allowed for this technique to be used for the first time in the characterization of artworks. The possibility of in situ analysis by the use of fiber optics or a microscope, allied with high sensitivity, was a significant advantage compared with techniques that required sampling. Renè de la Rie was the first to apply the potential of fluorescence spectroscopy for the characterization of art materials [47-49]. In 1982, he approached the fluorescence of madder colors and individually studied alizarin and purpurin. He noticed the increase of fluorescence when aluminum hydroxide is added to a solution of purpurin, thus describing the emission properties of a lake pigment [47].

In 1990, Arie Wallert, to overcome the difficulties of analyzing manuscripts and the lack of samples available, used fluorescence and absorption spectroscopy complementarily in the characterization of dyestuffs obtained from brazilwood, *folium*, lac dye, orchil, among others. He also analyzed the colorants by making three-dimensional analysis, in which it was possible to make a sequence of emission spectra at successively fixed excitation wavelengths [30]. Wallert found that the use of a three-dimensional representation allowed the identification of the colorants tested, although only analyzing distinct classes of dyes, i.e., neoflavonoids, anthraquinones, phenoxazones, among others, not exploring similarities between colorants of the same class [30]. Later on, in 2007, Maarten van Bommel and co-workers, also applied the three-dimensional analysis to the study of early synthetic dyes (1850-1900). The system used was a spectrofluorimeter with a fiber optic system, suitable for the direct analysis of embroideries from the Van Gogh Museum [50]. The authors found that the maximum fluorescence peaks are not sufficient for the identification of synthetic dyes, and they considered the excitation maximum as well [50]. They also found difficulty in understanding if a fluorescence pattern arose from a single species, or from a more complex mixture. Moreover, if a chromophore was present in very low amounts or it had low fluorescence quantum yield, it was invisible in the 3D-plotting [50].

The group currently led by Miliani, in Italy[13], have contributed significantly to the developments of fluorimetry applied to the analysis of cultural heritage. As part of the IPERION CH[14] project, they developed the MOLAB LABoratory, a network of facilities from several countries providing access to a set of portable equipment for the analysis of artworks, in response to the growing interest in the use of techniques that do not require microsampling. In 1998, Miliani, Romani and Favaro analyzed two main classes of organic colorants, anthraquinones, and indigoids, by absorbance and fluorescence spectroscopy. The group compared, for the fluorescence measurements, a front-face geometry with the application of a fiber optic accessory. They found that the two distinct instrumental set-ups were

[13] The research group in Cultural Heritage Science in Perugia is a collaboration between the Università degli Studi di Perugia and the Consiglio Nazionale delle Ricerche.

[14] IPERION CH is a consortium of 24 partners of a unique European research infrastructure for Cultural Heritage. It offers, among others, access to a wide range of high-level scientific instruments. For more information see http://www.iperionch.eu/. Previously, the group at Perugia coordinated the CHARISMA European Project (Cultural heritage Advanced Research Infrastructures: Synergy for a Multidisciplinary Approach to Conservation/Restoration), derived from the EU-ARTECH project (Access, Research and Technology for the conservation of the European Cultural Heritage), from which MOLAB was developed, in 2004.

substantially equal, and that fluorimetry allowed for the identification of the colorants, while absorption gave a more ambiguous indication [51].

During the 2000s, the group extensively applied fluorimetry, allied with absorbance spectroscopy, to the study of the chromophores present in colorants used between Antiquity and the 20th century. Alizarin and purpurin were studied in function of pH [52] and in the presence of Al^{3+} [53]. Romani and co-workers found that the emission spectra of both antraquinones varied significantly, with structureless broad bands for alizarin and a structured spectra for purpurin metal complexes [53]. Favaro and co-workers also studied the quantum yields and lifetimes of carminic acid and carminic lakes, both in solution and on painted surfaces [54], as well as the separation by HPLC and characterization by spectrophotofluorimetry of six components from saffron [55]. The group found that the emission bands were broad and it was visible some overlap among spectra of different species, both for the various components of saffron, as well as for pH dependency of carminic acid [54, 55]. Later on, Favaro and co-workers also approached the aging of madder dyed wool by chromatographic and spectrometric techniques [56] and the characterization of luteolin and apigenin [57]. Similarly to previous studies, the group described the transformation of weakly fluorescent molecules to efficient fluorophores in the presence of metal ions (Al^{3+}) [57]. Romani and co-workers also described the same effect while studying lake pigments, noticing also the presence of self-absorption effects which required correction by the Kulbelka-Munk theory. This is described in more detail in **subchapter 1.1.4** [58, 59]

In 2006, the group at Perugia developed a portable instrument suitable to record fluorescence spectra on surfaces using fiber optics, and in 2009 it was tested in the identification of orcein and indigo in Renaissance tapestries [60-62]. The technique proved to be valuable for the in situ analysis of artworks, although requiring the use of complementary experimental approaches. In 2008, Romani and co-workers developed an equipment for luminescence lifetime measurements. This portable instrument, based on time-correlated single-photon counting technique[15], was purposely designed for in situ measurements of luminescence lifetimes on artwork surfaces [63]. The group intended to surpass the disadvantage of spectrofluorimetry in the UV-Vis, such as the broadening of the emission spectra, which impedes the distinction between fluorophores with overlapping emissions. While some organic colorants may present similar emission spectra, their fluorescence lifetimes can be notably different [63]. These techniques, as part of the MOLAB project, belong to a multi-technique approach for the in situ analysis of artworks [64]. This approach was tested in several projects for the study of artworks, from paintings [65], manuscripts such as the Book of Kells [63, 66], an illuminated 16th century Persian manuscript [67], several pre-Columbian codices [68-70], and dozens more artworks [63, 64, 71]. More recently, in 2015, for the understanding of photoluminescence data, the group used theoretical predictions, DFT/TDDFT (density functional theory / time-dependent density functional theory) for the study of the absorption and fluorimetric properties of alizarin dyes [72]. The main characteristics of the portable fluorimetry instrument used by Miliani and co-workers are described in **Table 1.1**, and further information can be consulted at [62-64].

[15] Time-Correlated Single-Photon Counting (TCSPC) is the detection of single photons and the measurement of their arrival times in respect to a reference signal, usually the light source [63].

The works by the group of Perugia proved to be valuable for the application of fluorimetry in the characterization of organic colorants. They found that reliable identification of the chromophores could be achieved when fluorescence spectroscopy is coupled with other complementary techniques [63]. The group also found that the technique is capable of distinguishing between organic colorants if a suitable database is available [60]. Furthermore, this technique may help in the selection of appropriate areas for micro-sampling [61]. However, the 2 mm^2 spatial resolution prevents the in-depth characterization, such as the case of various colored threads in a tapestry [61], and distortion effects may occur, due to self-absorption, discussed further in **subchapter 1.1.4** [58]. The lack of structured spectra in some cases, such as those of low emitting species, e.g. alizarin, prevents the identification of the chromophore, providing clues only on the class of the natural colorant [65, 67, 68].

Another group from Italy, Aceto, and co-workers, have also applied fluorimetry in the characterization of artworks. The group used spectrofluorimetric analysis, together with fiber optic reflectance spectroscopy (FORS), both portable instruments, for the study of purple dyed manuscripts, such as *Codex Brixianus*, and the Coronation Gospels [73-76]. They have also characterized purple dyes by a multi-analytical approach of both bench-top and portable instruments, which included fluorimetry, FORS and TCSPC [75-77]. For more information on the equipment, please see **Table 1.1**. The use of a fixed wavelength source (LED's) can lead to the contribution of other materials, such as the case of parchment when using a LED centered at 365 nm [73, 75]. These contributions lead to increase difficulty in interpreting fluorescence spectra of organic colorants, as seen in the analysis of purple dyes, orchil and *folium*, by Aceto and co-workers [75]. Furthermore, they also acknowledged the influence of the chromophore's concentration in the fluorescence spectra, causing distortion effects, as discussed previously [75, 76]. Consequently, the group stated the need for complementarity between fluorimetry and reflectance spectroscopy for the characterization of similar colorants [75]. The similarities of fluorescence maxima of orchil and *folium* in solution (592 nm and 595 nm, respectively) [76], and the influence of the support in dyed samples as well as the presence of self-absorption effects [75, 76], prevents a complete and unequivocal differentiation of these purple dyes in artworks. Moreover, the unknown molecular structure for *folium*, and the lack of a robust database of references, considering that orchil could have been produced by several lichen species, may cause erroneous or inconclusive identifications.

In 2014, Mounier and co-workers from the CNRS allied several techniques that do not require microsampling, such as hyperspectral imaging, XRF, FORS, and fluorimetry, for the study of fragile objects for which sampling is prohibited [78]. The group mainly used fluorimetry for the identification of red pigments and organic binders, analyzing only two natural colorants, cochineal and brazilwood [78]. Later on, the group designed and patented a new portable, lightweight spectrofluorimeter device using ultraviolet LEDs sources (LEDμSF) within the framework of a project studying medieval illuminations [79]. The group tested and compared the equipment with bench-top spectrofluorimeters, one of those was coupled with a microscope, and found a good correlation between the data acquired [79]. More recently, in 2018, they used this newly developed portable equipment, combined with reflectance spectroscopy and XRF for the study of Japanese *ukiyo-e* prints [80]. In this study, the group mainly characterized red organic colorants belonging to different classes: brazilwood (neoflavonoid), safflower

and dragon's blood (flavonoids), and cochineal and madder (anthraquinones) [80]. The emission maxima ranged between 588 nm and 630 nm, with small shifts, such as the case for brazilwood and cochineal (625 nm and 630 nm, respectively). Therefore, complementarity with reflectance spectroscopy was necessary to provide identification of the organic colorants present in the Japanese *ukiyo-e* prints [80].

Mounier and co-workers, like Aceto, noticed the influence of other materials, e.g. the binder and support, when exciting the sample at low wavelengths with an LED source centered at 375 nm [80]. The group acknowledged that the use of LED sources at higher wavelengths, preferably at the maximum of excitation, may be more suitable for the characterization of red organic colorants, such as safflower [80]. Because of the use of LED's centered at low wavelengths, instead of the maximum of excitation, low emitting fluorophores may require longer integration times for analysis, such as the case of alizarin. For more information on the equipment, please see **Table 1.1**.

Other techniques, such as laser-induced fluorescence (LIF), fluorescence multispectral imaging (fMI) and fluorescence lifetime imaging (FLIM), have been applied by Comelli, Nevin, and co-workers, for the analysis of artworks. fMI requires the acquisition of emission at different excitations [81], while FLIM, measures the temporal properties of fluorescence in several points of the artwork, providing a reconstruction of the lifetime map of the region that is analyzed [82-86]. FLIM and fMI were combined with other techniques, in the analysis of a Renaissance wall painting by Masolino da Panicale, enabling the identification of an anthraquinone-based lake pigment [81, 87]. The same group has also developed time-resolved photoluminescence spectroscopy (TRPL), for the analysis of art materials [87]. This technique measures the evolution of the luminescence spectrum over time, also with a pulsed laser source. These techniques, LIF, FLIM, and TRPL, haven't been commonly applied on the characterization of organic colorants due to the complexity of the data, requiring complementary elemental and molecular analyses [87].

Other groups, namely in the USA, have also applied fluorescence microscopy to the analysis of artworks. Wustholz and co-workers have developed a novel approach for the study of organic materials in microsamples, using SERS coupled with fluorescence microscopy [88]. In this methodology, SERS and fluorescence measurements are performed on an inverted microscope, using a 632.8 nm HeNe laser for Raman and a 532 nm excitation for emission [88]. The group showed a good correlation between the SERS identification and the fluorimetry measurements, however, the methodology can only be applied on microsamples available for SERS analysis. Moreover, it is necessary to further understand the pigment-nanoparticle interactions, which may affect fluorescence spectra.

Delaney and co-workers have applied luminescence imaging using a camera system to obtain both reflectance and luminescence images, allied with FORS and XRF, for the identification of paint materials of Picassos' Harlequin musician, and on a choir book of the Compagnia di Sant'Agnese [89, 90]. However, although natural colorants were found, the identification of the dyes present was not possible by luminescence imaging.

In Japan, Shimoyama and co-workers applied EEM contour plots (excitation-emission matrix, with fiber optics) in the characterization of artworks, namely of votive figures of Japanese culture, identifying indigo blue [91]. Sasaki Y., Sasaki K. and co-workers have also used this technique for the characterization of textiles [92, 93]. The group identified yellow dyes in braids used in Japanese armors [92], and common red and yellow colorants used in Japanese textiles, such as safflower, Japanese madder, and sapanwood and protoberberines, by combining EEM with reflectance spectroscopy [93, 94]. In the same way, Nakamura and co-workers were able to identify several dyes, such as safflower, in ancient Japanese textiles, using a spectrofluorimeter with fiber optics acquiring EEM contour plots [95, 96]. Although providing both excitation and emission data, the set-up used for the EEM (with a fiber optic, hence a spatial resolution in the order of the millimeters) may lead to the appearance of distortion effects, such as self-absorption and scattering. Nakamura and co-workers extensively reported these phenomena during the characterization of ancient textiles, which can, occasionally, prevent dye identification [95, 96]. For more information, see **Table 1.1**.

Soltzberg and co-workers and Derrick, Newman & Wright also applied EEM (with fiber optics) for the characterization of synthetic organic dyes and Japanese *ukiyo-e* prints [97, 98]. Soltzberg and co-workers found the technique to be highly sensitive and robust, although taking into account the influence of instrumental artifacts such as scattering, which may influence the interpretation of the data [97]. On the other hand, Derrick, Newman & Wright found that, although it was possible to assign contour plots to most colorants, some had multiple possible identifications due to similarities [98]. When considering the variations of signals from the same colorant due to differences in production, the interpretation of EEM contour plot database may become challenging. Moreover, low emitting fluorophores, such as alizarin, show virtually no fluorescence peaks, hindering their identification.

Melo and co-workers, at the DCR FCT NOVA, Portugal, developed in 2008 a methodology for the study of organic colorants in artworks. The group explored, for the first time, confocal microspectrofluorimetry for the characterization of red lake pigments, as a response to the lack of techniques for the analysis in-situ, or in small samples from artworks [16]. The group found that the rapid acquisition of spectra, allied with high spatial and spectral resolution, makes this technique very powerful for the characterization of organic colorants, allowing the analysis of individual pigments particles or aggregates in a paint film. The data is reliable and reproducible, and a semi-quantification for madder lakes was tested and proven successful [15, 16].

The spectrofluorimeter used was coupled with an Olympus BX51M confocal microscope, which allowed the spatial resolution to be controlled by multiple-pinhole turret (pinholes 1–10 corresponding to 2–60 μm spot with a 50X objective). Steady-state fluorescence spectra were acquired using a continuous 450 W xenon lamp, which is directed into a double-grating monochromator. Monochromatic light from the monochromator is fed across a spectrofluorometer-microscope interface. The incident excitation beam is directed onto the sample, which is resting on a programmable x−y−z microscope stage. Fluorescence from the sample is directed back up into the microscope. Beam-splitting is obtained with dichroic filters used at 45º; they reflect the short wavelengths (excitation) and transmit the long wavelengths (emission) [16]. See **Figure 1.7** and **Table 1.1**.

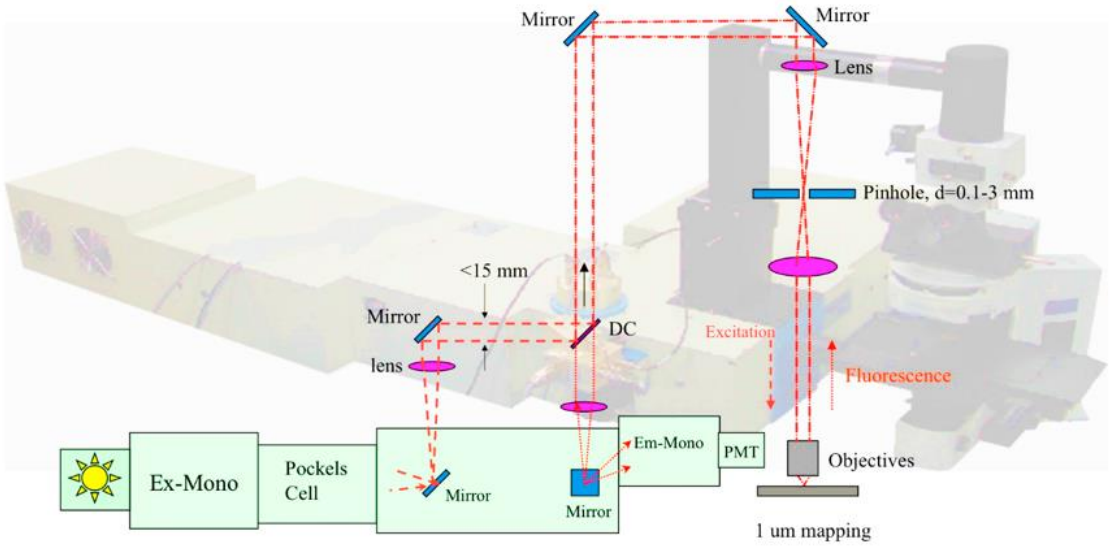

Figure 1.7. Scheme of the microspectrofluorimetry apparatus, based on [19].

The technique was tested on 19th-century reconstructions [16] and validated in the study of medieval illuminations, paintings cross-sections, and textiles [15]. In 2010 it allowed the identification of the red colorants in Van Gogh paintings and ancient Andean textiles, proving the versatility of the technique [17]. Since then, it has been frequently used for the identification of red organic colorants in medieval manuscripts [18-20, 25-27] and paintings [15, 17].

Microspectrofluorimetry, although being a bench-top equipment offers the possibility to acquire both emission and excitation, as seen by Shimoyama, Sasaki, Nakamura and Newman, with the advantage of a coupled microscope. It is highly sensitive and selective, allowing for the excitation at a desired wavelength, enabling the characterization of a mixture of fluorophores. This technique will be further explored in this doctoral project.

Considering the research groups using fluorimetry for the analysis of dyes in artworks, it is clear the variability of set-ups available. The differences are not only on the excitation source, but also on the detector, the probe used and spectral and spatial features. The comparison between the main set-ups described can be consulted on **Table 1.1**, and the advantages and disadvantages are further discussed in **subchapter 1.1.4**.

Table 1.1. Description of the set-ups used by main groups currently applying fluorimetry in the analysis of natural colorants in artworks. Other complementary techniques, such as reflectance spectroscopy and TCSPC are described if used simultaneously and complementary to fluorimetry. §

	Type	Techniques	Spectral range (nm)	Source	Detector	Probe	Spectral resolution	Spatial resolution	Probe-artwork distance	Ref.
Melo *et al.*	Bench-top	Fluorescence & Excitation	400 - 700	450 W xenon lamp	Photomultiplier	Olympus BX51M confocal microscope	2 nm	2 – 60 µm multiple-pinhole turret	Confocal microscope	[15, 16]
Miliani *et al.*	Portable	Reflectance Fluorescence TCSPC	200 - 1600 300 - 1600 350 - 850	Deuterium-halogen lamp LED (445 nm (50 mW)) and diode (375 (18 mW) and 640 nm (100 mW))	CCD Photomultiplier	quartz fiber optic 20º silica fused fiber optic	2 nm 25 nm 100 ps	10 mm 2 mm 12 mm	2 mm 4 mm 4 mm	[62-64]
Aceto *et al.*	Portable	Reflectance Fluorescence	360 - 1100 191 - 886	Tungsten-halogen lamp (360-2500 nm) LED source (365 nm)	CCD	quartz fiber optic 45º Vis/NIR fiber optic	2.4 nm 7.6 nm	3 mm 1 mm	1 mm 1 mm	[73-77]
Mounier *et al.*	Portable	Fluorescence & Excitation	200 - 1000	LED sources (375 nm (5 mW) and 285 nm (300 µW)) Density filters may be used	CCD	fiber optic	2 nm	1 – 2 mm	4 cm	[79]
Nakamura *et al.*	Bench-top	Fluorescence & Excitation EMM	300 - 700	Xenon flash lamp	n.a.	quartz fiber optic	1 nm	4 mm	n.a.	[96]
Newman *et al.*	Bench-top	Fluorescence & Excitation EMM	200 - 900	15W xenon flash lamp	Photomultiplier	quartz fiber optic 45º	5 nm	2 – 3 mm	1 mm	[98]

§ - In lighter grey is marked the equipment used in the doctoral project.

1.1.4. Advantages and disadvantages of different spectrofluorimetric set-ups

Techniques based on UV-Vis luminescence have been reported to present shifts in emission bands related to physical interactions with the radiation emitted by a fluorophore. These interactions can be expressed as self-absorption or multiple scattering [99]. Consequently, the observed emission spectrum may differ considerably from the intrinsic fluorescence, by reduction of the emission intensity, red shift of emission maxima, or even distortion, making impossible the identification of an unknown sample.

While dilute solutions should be unaffected by self-absorption, multiple scattering, and aggregation phenomena, allowing the intrinsic fluorescence of the lake to be measured; this is not the case for concentrated samples [100]. Miliani and co-workers found, while studying cochineal lake pigments, that the more concentrated the dye, the farther the emission maxima is from the intrinsic fluorescence in solution leading also to reduction in emission intensity [99]. The group explained this phenomenon with the increase in absorption at high concentrations, leading to an overlap of absorption and emission profiles. The photons emitted by the lake are absorbed by the fluorophore itself leading to profound modifications of the intrinsic fluorescence spectrum, shifted towards longer wavelengths. Not only, but the emitted photons can also undergo multiple scattering, where the radiation emitted inside the paint layer at shorter wavelengths experiences more scattering, before reaching the surface than that at longer wavelengths [99]. The group applied the Kubelka-Munk model to obtain a corrected emission spectrum, similar to those acquired in dilute solutions [99, 101]. This model defines that for a solid sample, it is possible to define a re-emission function *F(Rλ)*, at each irradiation wavelength *(λ_0)* as a function of the experimentally determined reflectance *(R)* values:

Equation 1.1

$$\frac{k(\lambda)}{s(\lambda)} = \frac{(1-R_\lambda)^2}{2R_\lambda} = F(R_\lambda)$$

where *s(λ)* represents the ideal scattering coefficient, *k(λ)* is the ideal absorption coefficient, and R_λ is the diffuse reflectance at the corresponding wavelength λ. Such function is used for the correction factor calculation shown in **Equation 1.2:**

Equation 1.2

$$\gamma(\lambda,\lambda_0) = \frac{1}{1+\sqrt{\frac{F(R_\lambda)}{F(R_\lambda)+2}}} \frac{1}{1+\sqrt{\frac{F(R_\lambda)(F(R_\lambda)+2)}{F(R_{\lambda_0})(F(R_{\lambda_0})+2)}}}$$

where λ and λ_0 stand for the emission and excitation wavelengths, respectively. The γ parameter, obtained at each emission wavelength, considers the self-absorbed light so, the ratio between the experimental emission spectrum and the γ parameter provide the true emission spectrum, corrected by light re-absorption [58, 102].

The Kubelka-Munk model has to be used with caution. It was developed for homogeneous samples, and only the diffused light should be collected, which is experimentally impossible due to the

heterogeneity of a painted layer [102]. Moreover, the description of the paint layer as a homogeneous dispersion of pigmented particles in an organic binder is an oversimplification of real cases [99].

As seen in **Table 1.1**, fiber-optics are extensively used as a probe for the emission measurements of natural colorants in artworks, and due to the spatial resolution in the order of the millimeters, it is common to encounter many of these distortion phenomena [58, 99, 102]. However, in the case of spectrofluorimetry coupled with a microscope, these effects do not occur, since it simulates a diluted solution [16].

The lamps used and the detectors also affect the type and quality of the information obtained. In **Table 1.1** it is visible that the source used to excite the sample varies considerably. Tungsten lamps are usually used to measure the visible and near-IR regions (350-2500 nm), while the deuterium lamp is used to measure in the UV (185-375 nm) [34, 103]. The most common lamp for fluorescence measurements is the high-pressure xenon arc. This lamp presents one main advantage regarding other light sources: it provides a continuous output over the range of 250-750 nm. Furthermore, they are considered as being ozone-free, meaning that they do not generate ozone while operating [34, 103]. Xenon flash lamps can also be used, as seen by the group of Nakamura. LED (light-emitting diodes) are more recent light sources for spectrofluorimeters, and a wide range of wavelengths is already available. The excitation wavelength is better defined by the use of filters, although one is limited by the available LED excitation wavelength [34, 103].

Several detectors are also available. A photomultiplier tube works in the 160-1100 nm region, while CCD's (charge-coupled devices) work in the 200-1100 nm region. The first is suitable for photon-counting mode because it responds to individual photons, while CCD's, on the other hand, are remarkably sensitive detectors, containing 10^6 or more pixels. Each pixel acts as a detector where charge accumulates in proportion to total light exposure [103].

Microspectrofluorimetry, although not being a portable instrument, offers other significant advantages, such as the possibility to acquire both emission and excitation, in-depth profiling and better quality in signal to noise ratio [16]. Moreover, the ability to excite at the desired wavelength, on account of the use of a xenon lamp, enables the characterization of a mixture of fluorophores. However, portable equipment allows for the analysis to be carried out without the need to move the objects, which can be expensive, risky and in some cases, impossible. Some of these portable instruments, such as those used by Miliani and co-workers, offer the possibility to acquire fluorescence lifetime which may allow the distinction of different organic colorants having similar fluorescence spectra [62-64].

1.2. Research aim and methodology

This project is divided in two parts: in **Part 1** microspectrofluorimetry, explored by Melo and co-workers, is allied to statistical and discriminant methodologies, for a better interpretation of the signal provided; in **Part 2** this approach is applied on two case studies, the Ajuda Songbook and a set of Islamic manuscripts. Using chemometric modeling of the data acquired from the spectrofluorimeter, this doctoral project intends not only to identify the colorant present but also to address the complexity behind the color formulation. Recipes' particularities can provide insight into chronological and location specificities, enabling a better understanding of the making of the artists' materials and, inevitably, the artworks.

Statistical and discriminant methods were already employed with success to discriminate between art materials, namely by Aceto and co-workers, using reflectance data of pigments [104-106]. The group was able to differentiate between blue pigments present in miniature paintings, using principal component and cluster analysis [104-106]. For this approach, reflectance spectra were acquired along side with XRF measurements and Raman analysis [104, 105]. Considering the experimental set-up, the differentiation of azurite, indigo and ultramarine by FORS does not appear to add crucial information since all pigments are easily distinguishable by Raman and XRF. In fact, classification had to be performed among colors of the same hue, since if all were considered, the discrimination would be predominantly for the hues, and not the colorants of similar hue. In the discrimination of natural and synthetic ultramarine, the same methodology was applied, in which it was suggested that the reflectance data should be transformed to avoid influence of the surface roughness and colorant concentration. The transformations suggested included apparent absorption ($\log\left(\frac{1}{R}\right)$), Kubelka-Munk and range scaling[16] [106]. For the application of the Kubelka-Munk, one must be cautious since it may not be representative of real cases.

The same methodology also supported the identification by FORS and XRF of different types of greens (pure greens and mixtures) applied in manuscript *Corale 43*, although not providing a conclusive distinction between copper based and green earth pigments [107]. Mounier and co-workers have also applied principal component analysis for the distinction by FORS of red organic and inorganic pigments: brazilwood, cochineal, cinnabar, red lead, and red ochre [78]. As stated by the group, the FORS spectra of the five red pigments are very different and characteristic, consequently principal component analysis (PCA) only confirmed the attribution.

Data from other techniques, such as ICP-MS (inductively coupled plasma–mass spectrometry) and DRIFT (diffuse reflectance infrared Fourier transform), have also been combined with multivariate statistical tools to successfully obtain differentiation between inorganic materials [108, 109]. In the first case, the combination of statistical methods with ICP-MS data provided important information on the geological markers of ancient pottery samples [108], while the combination of this methodology with DRIFT allowed the discrimination of five different classes (carbonates, sulphures, silicates/iron-oxides, lead-oxides and organics) [109]. Interestingly, although discriminating the classes of the pigments, the

[16] Range scaling is the change in coordinates according to the formula: $R_{rs} = (R_x - R_{min})(R_{max} - R_{min})$; where R_x is the original reflectance value and R_{min} and R_{max} are respectively the minimum and maximum reflectance values in the spectrum [106].

organic samples are more dispersed in the scores plot of PCA. On a different approach, Lopes and co-workers [110] used multivariate deconvolution techniques to discriminate between proteinaceous binders by infrared analysis. It is very difficult to unequivocally discriminate between binders, and the use of red and blue historically accurate reconstructions allied with PCA proved successful [110]. Later on, Mas *et al.* applied MCR-ALS (multivariate curve resolution alternating least squares) to infrared spectra for the characterization of proteinaceous binding media used in medieval paints [111]. Mas and co-workers use this methodology to quantify the binding media formulations, thus enhancing the knowledge on paint colors [111]. Previously, Nevin and co-workers had proven the potential for Raman spectroscopy, allied with PCA, for the identification and discrimination among protein-based binding media used in paintings [112, 113].

Moreover, the use of multivariate analysis has successfully discriminated between infrared data of historic blue pigments and blue tempera paintings commonly found in works of art [114], binding media used in painting [115] and between infrared and Raman data of household paint samples [116]. For the latter, several pretreatments were tested as well as several chemometric methods, allowing for the discrimination of the paints by type of binder using infrared analysis, and by organic pigment content using Raman analysis. Another use for multivariate analysis was explored in the discrimination of samples on the basis of the interaction between the pigments and the egg yolk-based binder [117].

To conclude, fingerprint techniques, e.g., Raman and infrared, when analyzed by statistical tools, enable the characterization of complex data, and the extraction of valuable information such as geographical provenances, chemical interactions and discrimination between compounds belonging to the same chemical class (binders, pigments). However, when applied to reflectance spectra, little information was extracted, except for the confirmation of what had already been pointed-out by other techniques. Moreover, the studies present thus far, with an exception by Mas *et al.* [111], were performed with principal component analysis and hierarchical cluster analysis, two of the most used chemometric techniques.

To the author's knowledge, multivariate statistical tools applied to fluorescence data have never been applied in data acquired from historical reconstructions of artists' materials or artworks. This type of in-depth information on the color formulation has never been addressed with this methodology. Previous studies, discussed in **Chapter 1.2.3**, focused on the identification of organic colorants or the class of fluorophores, i.e., anthraquinones, flavonoids, among others. This novel approach can provide insight into the making of organic colors which is crucial for the dating of artworks and the characterization of *scriptoria* or artists' workshops, see **Scheme 2**, **Synopsis**.

This approach developed in **Part 1** is subdivided into two parts: *i*) development of the methodology with a database of historical reconstructions, **Chapter 2**; *ii*) testing and validation with data acquired from historical artworks, **Chapter 3**. This work will contribute to the future development of an algorithm-based software which performs statistical modeling to generate a prediction, which will contain the colorant identification and other recipe' specificities.

Chapter 2 focuses on the development and testing of modeling strategies applied to the historically accurate reconstructions database of four natural red dyes and their lake pigments, used during the Middle Ages: lac dye, kermes, cochineal[17], and brazilwood. The fact that the first three belong to the same class of colorants, anthraquinones (as seen in **Figure 1.1**), presents a challenge for their identification by fluorimetry. Multivariate statistical tools, i.e., Hierarchical cluster (HCA) and principal component analysis (PCA), as well as soft independent modeling of class analogy (SIMCA), were explored, with the spectral data acquired (both excitation and emission spectra). It is intended not only to discriminate between the four colorants but also to address specificities of recipes belonging to the same colorant.

In **Chapter 3**, the complexity of naturally aged color paints, such as those found in artworks is addressed and the methodology developed for the database of historical reconstructions is applied. The red colorants selected for this study, lac dye and brazilwood, were identified in artworks by molecular fingerprint techniques[18]. For both colorants, an extensive database of medieval manuscripts (11th – 15th c.) has been investigated during the past decade at the DCR FCT NOVA (155 spectra) [15, 17, 118]. The methodology developed with data from real artworks for lac dye and brazilwood will allow to explore the distinctiveness of the *scriptoria* or artists' workshops, enabling to pinpoint geographical and temporal particularities in the production of color paints, see **Scheme 2**, **Synopsis**. In **Appendix A.1.2**, the methodology is applied to cochineal, lac dye and brazilwood. Because cochineal has rarely been identified in medieval manuscripts, Andean textiles belonging to the MFA-Boston collection were used (4 samples, 24 spectra), since the chromophore present, carminic acid complexed with a metal ion, is what we would expect to find in other artworks.

This methodology will enable a deeper understanding of the making of the colors and paint formulations present in historical artworks, contributing to advances in art technological source research and better-informed decision-making processes regarding conservation treatments. The methodology developed in **Chapter 2** and **3** will be further put to use and tested in case studies, **Chapter 4** and **5**. The study of two sets of manuscripts, allow this approach to be tested in very diverse typologies from different ambiences.

The first case study is the **Ajuda Songbook**. Galician-Portuguese songs, or *cantigas*, are among the most beautiful and characteristic productions of medieval Iberian cultural heritage. Composed and sung in the royal and noble Iberian courts roughly from the end of the 12th to the middle of the 14th century, Galician-Portuguese songs were collected and transcribed by the middle of the thirteenth century. Songbooks, or *chansonniers* in French and *cancioneros* in Spanish, represent the need for collecting oral poems into codices, where a song, made permanent in a manuscript, tells a story [119]. Altogether, we have access to some 1680 secular or courtly songs written by some 160 troubadours (*trovadores*, noble) and minstrels (*jograis*, non-noble) who were born in different Iberian kingdoms and territories, but all of whom composed in the language of the peninsular northeast [119]. The *cancioneros* were ordered

[17] Due to the lack of medieval recipes for cochineal, 19th century recipes from the Winsor and Newton archive were used.
[18] Kermes has not been identified in artworks by Melo's team thus far, and so, it is not included in the methodology developed for the second part of this study. The comparison of the colorants in **Chapter 3** with cochineal is discussed in **Appendix A.1.2**.

by genres and authors and are very diverse in content, reflecting the taste of the courts in which they were compiled [119].

The known existing testimonies of this Galician-Portuguese tradition, transcribed between the 13th and 16th centuries, are scarce and incomplete: the Ajuda Songbook (*Cancioneiro da Ajuda*), the National Library Songbook, or Colocci-Brancuti Songbook (*Cancioneiro da Biblioteca Nacional*, BNP), and the Vatican Songbook (*Cancioneiro da Vaticana*, Vatican Library), both from the 16th century [80]. In addition to these three large troubadour collections are two medieval manuscripts that include Galician-Portuguese songs: the late 13th c. Vindel Parchment (New York, Pierpont Morgan Library, MS M.979), which consists of seven songs by Martim Codax, six with music, and the 14th c. Sharrer Parchment (Lisbon, Torre do Tombo, ANTT Fragmentos, cx. 20, n° 2), which preserves seven love songs by King Dinis of Portugal, all with music, **Figure 1.8** [120].

Since the Sharrer and the Vindel parchments have reached us as fragments, the Ajuda Songbook is the only Iberian medieval codex, while the others are copies, made in Italy, of an older songbook, now lost, **Figure 1.8**. The oldest one of the three codices is, therefore, the Ajuda Songbook, of which there is no information on who commissioned it, its date, the location of its production, or the reason why its text, music, and illuminations were never finished, presently at the Ajuda Library (*Biblioteca da Ajuda*).

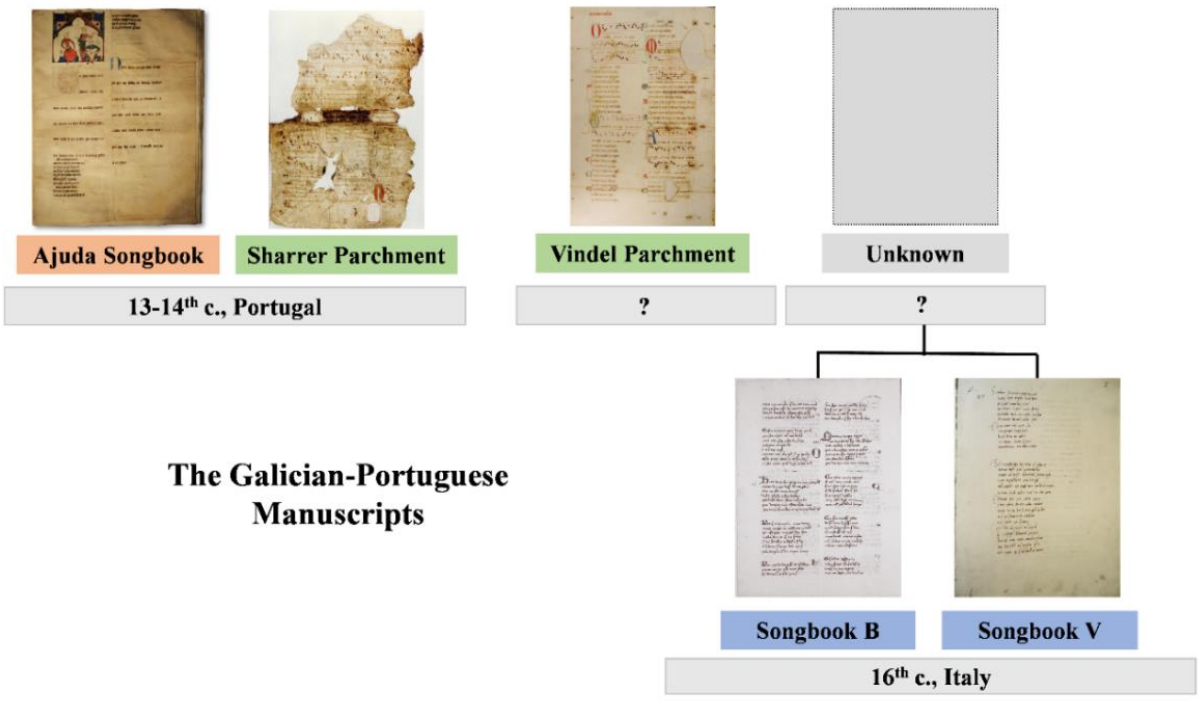

Figure 1.8. Galician-Portuguese secular songs and codices. Scheme adapted from the proposal of project STEMMA.

Nascimento once stated that "*as an isolated testimony of a lost tradition with no precise links in time, the Ajuda Songbook constitutes a monument to which becomes necessary to return, for we haven't yet fully understood its course"* [121][19]. This research work aims to advance our knowledge not only of this particular codex but of troubadour culture in medieval Iberia more broadly. The scientific analysis of the materials used to produce the Ajuda Songbook's illuminations will help to clarify the questions regarding the manuscript's date and other circumstances of its production. Within a collaborative and multidisciplinary approach, gathering experts from conservation science, art history, technical art history, literary studies, and music history, this study aims to fill in some of these gaps in scholarly knowledge about the Ajuda Songbook. Graça Videira and Luís Correia de Sousa bring their expertise in art history, literary studies and musical iconography, while the team led by Maria João Melo, with Paula Nabais and Rita Castro, supports this knowledge with the molecular characterization of the materials.

Our second case study is a group of **Islamic manuscripts** rescued from Timbuktu, Mali. Timbuktu was an important cultural center in Northern-Africa for manuscript production, with books brought in and out to other important cities such as Cairo and Mecca [122-124]. Timbuktu met its apogee during the 15th and 16th centuries, with more than 150 universities, becoming a center of academic studies in Northern-Africa. By the end of the 16th century, Timbuktu lost its importance, due to the invasion of the Moroccan army, which sacked the city and took the scholars to Fez [122-124].

The manuscripts studied were stored in libraries in and around the city of Timbuktu, until 2012, when a separatist faction of the Tuareg tribe tried to impose the Sharī'ah law[20]. Many historic buildings were destroyed, and this meant that the manuscripts, essential testimonies of the forgotten city, were at risk. The media and literature describing this event, report that a librarian, Abdel Kader Haïdara, organized a plan to rescue more than 370 000 manuscripts from 45 different libraries, through the course of several months [122-124]. Some manuscripts were carried through the desert inside metal cantines in rented 4x4's, while others were carried in boats down the Niger. Unfortunately, Abdel Kader Haïdara was not able to save around 4 000 manuscripts which were burned by the Tuareg faction, just before the French army took the city [122-124]. Although the librarian took some care in the preservation of the manuscripts, e.g., the safe houses to which the manuscripts were transported were equipped with air-conditioning or dehumidifiers, these works show severe signs of degradation due to exposure to extreme conditions.

[19] "*…pelo que representa como testemunho isolado de uma tradição sem elos precisos no tempo, o Cancioneiro da Ajuda constitui monumento a que se torna necessário voltar sem medir o tempo disponível, pois ainda não atinámos totalmente com o seu percurso*" [121].

[20] The Sharī'ah law, or Sharia, is an Islamic rule of right action, is an individual interpretation of the Koran and the Hadith (stories of the Prophet Muhammad's life). It is the set of religious principles of which family life, acts of worship and other aspects of daily life should follow. In Timbuktu, the faction banned music and other artistic and cultural representations, destroying mausoleums, palaces and libraries.

Figure 1.9. Islamic manuscripts studied within the scope of this project.

A recently financed project by the Spanish Ministry of Economy and Competitiveness allowed for the study and preservation of a group of manuscripts, probably produced in Al-Andalus. The project, IMAN (*Investigación y análisis para el conocimiento y la preservación de un patrimonio documental: los manuscritos andalusíes*)[21], selected six manuscripts, of which five[22] are present in this thesis: a Koran (1198), a Theology Treatise (14th c.), a Book of Poems from Al-Sarishi (15th c.), a Biography of the Prophet (1468) and Manuscript 19 (1485), **Figure 1.9**. The manuscripts are currently kept at the Timbuktu Andalusian Library (Biblioteca Andalusi de Tombuctu, Fondo Ka'ti). This project proved to be an excellent opportunity to study the materials and techniques used to illuminate these medieval books, focused on the molecular characterization and degradation assessment of the red dyes, as an excellent opportunity to test the methodology developed in **Chapters 2** and **3**.

For the analysis of cultural heritage, no technique alone can solve the complex problems encountered, whereas the synergistic combination of several techniques allows for in-depth characterization of artists' materials, required for a well-designed preservation or conservation intervention. Therefore, for the analysis of our two case studies, the technique explored in this doctoral project, microspectrofluorimetry, is allied with *i)* a robust database of historically accurate reconstructions, and with *ii)* a multi-analytical approach for the disclosure of the molecular palette, which is henceforth described.

[21] Financed by the Ministerio de Economía y Competitividad of the Spanish Government, HAR2016-77482-R.

[22] The sixth manuscript, a Treatise of Mathematics, was also analyzed. Because only ink was identified (no illuminations or other color paints), it was outside the scope of this thesis and therefore, the results are not presented.

1.2.1. Historically accurate reconstructions of lake pigments

As described in **subchapter 1.1.4**, regardless of the experimental set-up, fluorimetry does not have the fingerprint capability of a technique such as infrared spectroscopy, requiring the complementarity of other analytical methods, and a robust database, which has been developed by Melo and co-workers, as described in **Chapter 2** [18, 20, 25-27]. This database is mainly composed of historically accurate reconstructions of red lake pigments prepared following technical texts and recipes, complemented by the analysis of the artworks. As Oltrogge stated, "*our knowledge of artistic materials and techniques depends on two main sources: the scientific analysis of works of art and the study of written sources. In between stands the reconstruction, which in this context means the experimental execution of historical recipes*" [125]. These reconstructions help to understand the recipe and the material. Likewise, the reconstructions can also provide insight into degradation mechanisms, providing material for future research on conservation [125, 126]. On the other hand, art reconstructions depend on the availability of historical sources, which for the Middle Ages and Renaissance are scarce. Only a small number of treatises is available, which creates a gap in material and technical art history. Researchers following Merrifield's steps, who visited libraries to trace manuscripts and transcribe recipes, have contributed to making medieval texts available [127, 128]. The translations and critical edition of the *Liber diversarium arcium*, by Mark Clarke, is an example, as the Cologne database for painting materials and reconstructions, by Doris Oltrogge [125, 129].

Other approaches have also been developed, such as the new critical edition of the 'book of all color paints' which adds the experimentation on making reconstructions with as much historically accuracy as possible, to previous editions of the book [116]. This new critical edition gathers the knowledge obtained from a decade of systematic experimentation of the recipes in the 'book of all color paints,' allying it with the rationalization of the recipes and chemical reactions behind the phenomena, pointing out key aspects of the recipes [130]. Another example is the digitized Winsor & Newton database developed by Leslie Carlyle and Mark Clarke [127, 131, 132]

The importance of historically accurate reconstructions has led the group of Maria João Melo to study and reconstruct medieval processes for making paints and inks, for the past 15 years. The main approach was establishing a dialogue between the text and the molecular characterization: the recipes in medieval written sources and the multi-analytical molecular characterization of the original colors. The accuracy of the reconstruction is validated through comparison with the molecular analysis of the original colors of medieval manuscripts. If the molecular and morphology data of the reproduced color paint matches the original, it is validated and is included in the database. Thus far, red lake pigments have been explored to develop a robust database, to aid in the identification of organic colorants, which is the basis for the methodology developed in **Chapter 2** [15, 18, 20, 25-27, 132, 133].

1.2.2. Modus operandi: a multi-analytical approach

The approach developed in **Part 1** of this doctoral project envisages the increase of knowledge on how organic colorants were prepared, uncovering chronological and location specificities. However, when applied to case studies, as seen in **Part 2**, this approach is hand-in-hand with a multi-analytical methodology which foresees the characterization of the artwork as a whole: pigments, dyes, binders, additives and support. In this sense, the interdisciplinary team at DCR FCT NOVA has developed and tested an approach for the molecular characterization of colors in medieval manuscripts, of very diverse typologies, from the Romanesque Bible (12th c.) to the Books of Hours (15th–16th c.) [15, 118, 134]. This detailed molecular characterization is necessary to understand the conservation state of the medieval illuminations, which will contribute to better define conservation protocols.

The investigation led by this group was set in three axes: *i*) the creation of new methodologies for the characterization of historical dyes [15-20]; *ii*) study of the formulation of binders – the "glues" that fix the colorants for centuries – exploring statistical methods for the study of complex signals [110, 111]; *iii*) creation of databases of reference compounds [15-20, 25-27]. This investigation has been built in a permanent dialog with experts in history and medieval culture and the responsible for the preservation of these manuscripts in libraries and archives.

When studying medieval manuscripts, such as the case of the two case studies described in this doctoral project, all components are investigated, from the support to the color paint. The materials used to produce the latter include the colorant (dye and pigment), binding medium, and additives, see **Figure 1.10**.

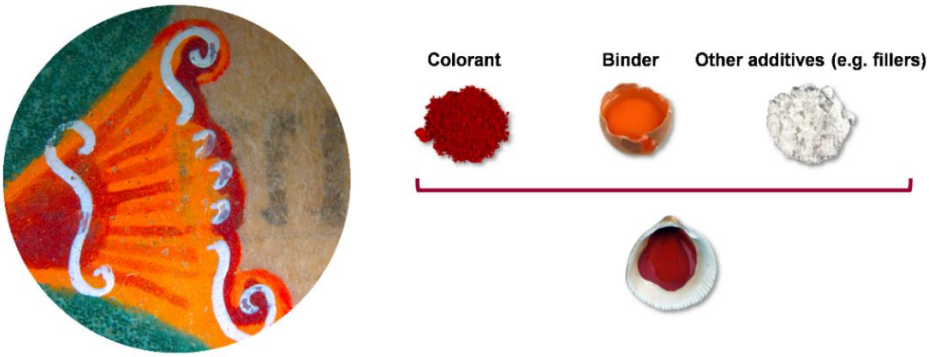

Figure 1.10. In a medieval color, we can find three main components: the colorant, the binding media, and additives such as fillers. Detail of a medieval paint from Lorvão 15, fol. 11.

When analyzing a medieval manuscript, the codex is first observed by optical microscopy to understand the built up of the color paint, make a first selection of the areas to be analyzed with more detail, and to identify possible degradation phenomena. Then, the molecular analysis of the color paints and inks begins, in a combination of spectroscopic techniques, allowing for the identification of all components. A first screening is done with micro-energy dispersive X-ray fluorescence (EDXRF), which enables the

identification of the elements present and allows a semi-quantification of pigment mixtures (by comparison with a reference database). Further characterization is performed by fiber-optic reflectance spectroscopy (FORS) in the visible region, and by combining Raman microscopy with Fourier transform infrared spectroscopy. While FORS allows for an indication of the colorant present, Raman and infrared spectroscopy provide a 'molecular fingerprint' – the unequivocal characterization of a single compound. For the identification of organic colorants, microspectrofluorimetry and surface-enhanced Raman spectroscopy (SERS) are combined. While microspectrofluorimetry allows the in situ acquisition of several data within the artwork, a single microsample analyzed by SERS can confirm the molecular fingerprint of the chromophore. This allows for higher quality information to be obtained, as well as a complete description of the paint [110, 111, 135-137]. Both microRaman and microspectrofluorimetry allow for high spatial resolution (1-5 μm spot), permitting the separate analysis of paint components [118].

Some techniques, such are the cases of Fourier transform infrared spectroscopy and SERS, require microsampling. The research conducted thus far has proven that microsampling minimizes risk and handling of the manuscript, which, in combination with in situ techniques, reduces the number of microsamples collected. This damage to knowledge-obtained ratio is considered highly favorable by conservators given the information obtained, providing information about degradation phenomena [138, 139].

This approach mentioned has been applied in several manuscripts studied, shedding light into the production of the Portuguese Romanesque *scriptoria* and the late medieval French books of hours in Portuguese collections. A group of 39 manuscripts was thus far analyzed from the monasteries of São Mamede of Lorvão (9), Santa Maria of Alcobaça (16) and Santa Cruz of Coimbra (14), held at the ANTT, BNP, and BPMP, respectively [20, 118, 135, 136-141]. Of the late medieval period, seven books of hours were studied, held at BNP and PNMAF [142-147]. The selection was made according to their chronology and on their artistic value. Other manuscripts have been studied, namely the Charter of Vila Flor (1512) and the Fernão Vaz Dourado Atlas [136, 148]. In **Figure 1.11** is presented the color palette of 12^{th} – 13^{th} century manuscripts to 14^{th} – 15^{th} century books of hours. The common pigments found in medieval Portuguese manuscripts are henceforth described.

The colorants found give insight into the manuscript chronology, and are, therefore, intrinsic to some typologies, such as the lac dye typical of the Romanesque bibles, and the brazilwood present in books of hours, **Figure 1.11**. For both case studies investigated within this doctoral work, the characterization of the entire palette, including dyes and pigments, will be very important, not only to establish chronologies, but to ascertain the conservation condition.

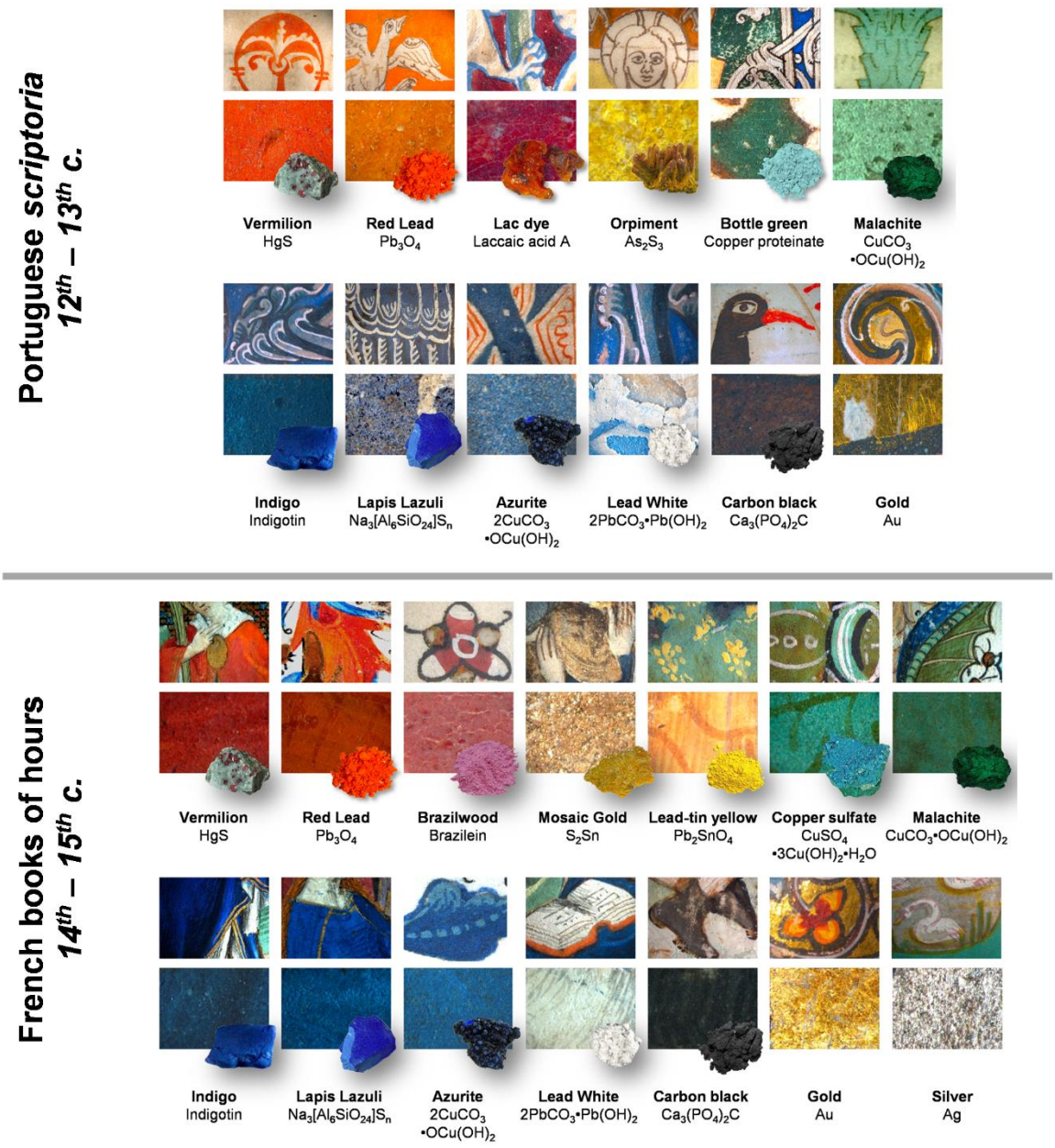

Figure 1.11. The color palette of medieval manuscripts: 12th – 13th centuries Portuguese *scriptoria* of São Mamede of Lorvão, Santa Maria of Alcobaça and Santa Cruz of Coimbra (*above*); 14th – 15th centuries French books of hours in Portuguese collections (*below*).

The blues could be produced with lapis lazuli, azurite, and indigo. Lapis lazuli is extracted from a semiprecious stone, a natural silicate of aluminum, sodium, and sulfur, with the formula $Na_8[Al_6SiO_{24}]S_n$, and it was highly praised due to its durability and excellent color, being imported from Afghanistan [149]. The color could range from different hues depending on the manufacturing process, namely the removal of impurities from the stone and the careful grinding to obtain a very fine pigment without losing its

color[23]. Azurite is a basic copper carbonate, with the formula $2CuCO_3•Cu(OH)_2$, also intense in color, although not comparable to lapis lazuli, and is usually considered a less expensive substitute. It needs the same careful grinding as the latter, or the artist would risk losing the mineral's color [149]. In medieval Portuguese manuscripts, the dark blues were produced by the addition of indigo to lapis lazuli, such as the case of the Santa Cruz manuscripts, or just by the use of indigo, such as the Lorvão manuscripts [136].

For the greens, the mineral malachite was commonly used, appearing in nature together with azurite. It is a basic copper carbonate, with the formula $CuCO_3•Cu(OH)_2$, and is very vivid in color. It was used from antiquity until the mid-19th c. although its use is more pronounced from the 15th century onwards [150]. Other greens were present, such as *verdigris*, which was used to "*indicate a number of different copper-containing compounds, including basic or neutral copper acetates, copper chlorides, copper carbonates, and other compounds*" and was the pigment of choice for 13th – 14th c. manuscripts [150]. Basic copper sulfates, such as brochantite, langite, antlerite and posnjakite, could also be found [142, 147, 150]. In Portuguese monastic illuminations, a deep saturated green was found, named *bottle-green*, a synthetic copper proteinate which was applied as a single color [118, 137]. Mixtures of yellow/blue pigments were also reported, such as *vergaut*. This mixture to produce green was mentioned in treatises such as the *Liber diversarum arcium*, where orpiment was used to create highlights that, together with indigo or black produced greens [129].

Red pigments also played an important role in medieval illuminations. Red lead and vermilion were the primary sources for the red color in manuscripts. The first, red lead (Pb_3O_4) is a bright orange pigment, also named *minium*, and is one of the first artificially produced pigments, by calcination of lead white [151]. Vermilion (HgS) was initially obtained from cinnabar, a mineral of mercuric sulfide. During the Middle Ages, the production of synthetic vermilion made this pigment more accessible to artists, and it slowly replaced red lead in medieval illuminations [149-152]. Red ochres were also used during the Middle Ages, although never reaching the importance of the red *per* excellence, vermilion [152]. Organic colorants were also extensively used, mainly the red (pink, carmine and dark red) and purple hues, as discussed previously and in **Chapter 2** and **3** [17, 118, 143, 153, 154].

Orpiment yellow (As_2S_3), a bright yellow, was extensively used in some of the oldest Romanesque manuscripts studied. Other yellow pigments appeared later on, such as mosaic gold (S_2Sn) and lead-tin yellow (Pb_2SnO_4), in the 14th – 15th c. books of hours [142, 147]. Yellow ochre, or goethite, although not common, it has also been found in medieval European illuminations. Finally, organic yellow colors although appearing to us nowadays as '*transparent traces*', were also used. Applied as an imitation of gold, such as the case of saffron (*Crocus sativus*), the fading and discoloration have turned these 'lost yellows' into a significant loss in medieval manuscripts [155].

Black and white colors were mainly used to darken on lighten certain hues, for contours or for creating volumes. Black was mainly produced by wood or bones ashes, or soot, such as the case of lampblack

[23] Lapis lazuli, if excessively ground becomes pale grey and loses its highly prized intense blue color. Muñoz Viñas notes that the use of this pigment is readily visible within a manuscript, because of the coarse size of the pigment particles [149].

(bone ashes, $Ca_3(PO_4)_2C$). This pigment was also extensively used as an ink or admixed with iron-gall inks [32]. The latter was highly praised for producing permanent writing vehicles [156, 157]. On the opposite of the brightness scale, white lead ($2PbCO_3•Pb(OH)_2$) was one of the most important pigments in medieval manuscripts and paintings [158]. Other materials, additives, such as gypsum ($CaSO_4•2H_2O$) or chalk ($CaCO_3$) could be mixed with the colorants to add “body” and turn the paint opaque without altering its hue.

Gold and silver were also extensively applied in medieval manuscripts, such as books of hours. Either in powder or leaf, they would add richness and splendor to an artwork, and so were highly valuable. However, silver, darkens with time, due to the formation of Ag_2S, which is becoming an increasing conservation issue in medieval manuscripts [147, 159].

Last, but not least, it is essential to discuss the binding media, the ‘invisible component,’ crucial for adhesion and, therefore, preservation of the paint layer [136]. Binders could be either polysaccharides, such as gum arabic, or proteinaceous, such as parchment glue, egg white (glair) or egg yolk. They could be applied as a single binder or as a mixture of two or more binders. These complex formulations play a role in the color as well as the paint’s film performance, such as viscosity, transparency, and permanence [136]. Studies show that in Portugal, protein temperas were used in the 12th and 13th centuries, while polysaccharide temperas or mixtures were used in the 15th century [20, 110, 111, 136].

The synergy between the multi-analytical approach developed for the analysis of medieval manuscripts and the new methodology for the study of organic colorants will be beneficial for the characterization of both case studies presented in **Chapter 3** and **4**. It will allow to identify the colorant present but also to get a better understanding of the paint formulation, which can be very specific chronological and geographically. The combination of this methodology with fingerprint techniques, such as Raman and infrared spectroscopies will allow to uncover all paint components, from the binders and additives to the chromatic palette.

The confocal spectrofluorimetry set-up available, as well as the expertise in the characterization of artworks, gathered by a multi-analytical approach and a robust database of historically accurate reconstructions, have placed this work in a privileged position to test the efficacy of multivariate data analysis on fluorimetric measurements. This approach will, for the first time, provide higher insight on the construction of color paints, well beyond the identification of the single fluorophore.

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Part 1. Microspectrofluorimetry for the safeguard of dyes and lake pigments

Detail from the Ajuda Songbook, 13th – 14th centuries: Musician, fol. 21 © Ajuda Library

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Chapter 2. Microspectrofluorimetry and chemometrics for the identification of medieval lake pigments

Abstract

Microspectrofluorimetry offers high sensitivity, selectivity, fast data acquisition, good spatial resolution (down to 2 µm), and the possibility of in-depth profiling. It has proved to be a powerful analytical tool in identifying dyes and lake pigments in works of art. To maximize the extraction of the information present in fluorescence emission and excitation spectra, we propose a chemometric approach to discriminate dark reds to pink colors based on brazilwood, cochineal, kermes and lac dye. These set of hues was obtained using a diverse range of medieval recipes for brazilwood, kermes and lac colorants and Winsor and Newton archive for cochineal lake pigments; the lake pigments were analyzed as color paints (arabic-gum and glair were the medieval binders selected). Unsupervised (HCA & PCA) and supervised (SIMCA) modelling were tested, allowing to explore similarities between colorants and classify the spectral data into the different lake pigments classes. It was possible to separate the four different chromophores based on their excitation spectra or by bringing together the emission and excitation spectra. The first method could also differentiate between the cochineal lake pigments, in particular between crimson lakes with different aluminates and an extender (gypsum) and between carmines with different complexing ions (aluminum and calcium).

2.1. Introduction

In the past few years we have been particularly interested in the development of methodologies that will promote a complete characterization of the organic colorants used in the past as well as their degradation products [1–12]. Changes in pigments, whether used pure or admixed, can alter the appearance of a painting significantly; consequently, the identification and state of degradation of colorants is of fundamental interest, since it provides critical information about the artists' aesthetic perspective, conceptions and choices, and how the work has changed over time. Therefore, it is desirable to develop methods that can characterize these materials directly on the artwork, in situ, or from small samples that may be available from works of art. Microspectrofluorimetry offers high sensitivity and selectivity combined with good spatial resolution and the possibility of in-depth profiling. It can also be used in situ without any contact with the sample or work of art to be analyzed, for movable objects that can be transported in the laboratory [13, 14]. The importance of sensitivity is clear when the following facts are considered: some of the dyes used in the past to create bright colors may have faded

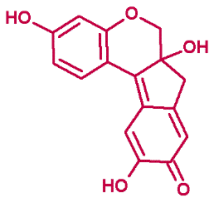
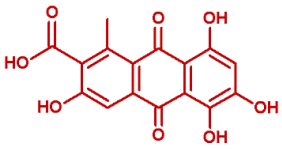
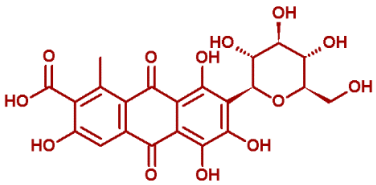
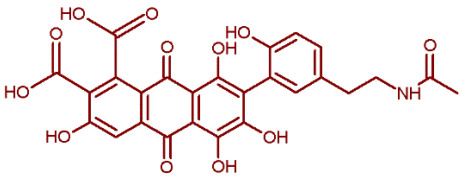
or may have been applied as very thin coats over, or mixed with, an inorganic pigment or extender, and therefore they may be present in very low concentrations. The possibility of in situ analysis of ancient colorants is a considerable advantage, particularly when considering that the techniques currently employed for dye analysis (HPLC–DAD-MS, microFTIR and SERS) require micro-sampling [15–17]. Microspectrofluorimetry also presents some drawbacks, namely the absence of a molecular fingerprint as disclosed in infrared spectra. This limitation may be overcome by combining surface-enhanced Raman spectroscopy (SERS), fiber-optic reflectance spectroscopy (FORS) and by using a consistent database build up with historically accurate reproductions of references for colorants, binders and color paints, which are the result of research into written sources of medieval techniques [13, 14, 17]. They are part of reproducing the process described in the source material as well as molecular identification and comparison with the original colors. This leads to a virtuous feedback loop, where reference compounds are validated against originals and are used to improve the analytical methods applied when identifying materials [11, 18–22]. A hypothesis that we will test in this work using a chemometric approach.

We will focus on four natural red dyes, and their lake pigments, used during the Middle Ages (found in medieval manuscripts and described in technical treatises): lac dye, kermes, cochineal and brazilwood, **Table 2.1**. The latter is a flavonoid, but the other three are anthraquinone reds extracted from animal sources, which makes their identification by an analytical technique such as microspectrofluorimetry very challenging.

Brazilwood has been extensively found in books of hours from the 15th – 16th c., and in the Galician-Portuguese Ajuda Songbook, possibly dated from the 13th c. [5, 11, 12] and is extracted from a tree, *Caesalpinia sappan* or other brazilwood species brought to Europe from Brazil from the 16th c. onwards (*Caesalpinia echinata*, *Caesalpinia brasiliensis*, *Caesalpinia violacea*, *Caesalpinia crista*, and *Haematoxylum brasiletto*) [18]. Kermes was obtained from a small insect, *Kermes vermilio*, found in the kermes oak, *Quercus coccifera* L. Other important historical sources of red derived from the resin secreted from the female lac insect, *Kerria lacca*, from which are obtained both the lac dye and the shellac resin. It was applied as a dark red or pink color in Portuguese manuscripts and it is characteristic of the Romanesque monastic production (12th – 13th c.) [1, 10]. In the 16th c. most of these sources were replaced by the red and scarlet colors of the American cochineal, *Dactylopius coccus*, commercialized by the Spanish empire [23]. Similar species were already found in Eastern Europe, *Porphyrophora polonica* and *Porphyrophora hamelli*, known as Polish cochineal and Armenian cochineal, respectively [23, 24]. In previous publications, we proved that confocal microfluorescence is a powerful tool for in situ analysis of colorants based on natural dyes [13, 14, 25].

Natural dyes may be described as weak to medium emitters. Following light absorption, an excited molecule is formed, and this fluorophore may lose its excess energy by emitting light. In a spectrofluorimeter, exciting at a single excitation wavelength and recording the fluorescence in the fluorophores' emission wavelength range results in an *emission spectrum*. It is also possible to excite at different wavelengths, following the colorant absorption spectrum, collecting at a single wavelength, obtaining thus an *excitation spectrum* that may reproduce the *absorption spectrum* [26].

Table 2.1. The four red colorants studied in this work, with the respective chromophores, provenience and chronology of occurrences in the Mediterranean world (in artworks).

Colorant	Main chromophore	Provenience	Chronology
Brazilwood *Caesalpinia* spp. *Haematoxylum brasiletto*	 Brazilein	Asia South-America	from 13th from 16th
Kermes *Kermes vermilio*	 Kermesic acid	Mediterranean Morocco Algeria	BC-15thc.
Cochineal *Dactylopius coccus* *Porphyrophora* spp.	 Carminic acid	South-America Eastern Europe	from 15th
Lac dye *Kerria lacca*	 Laccaic acid A	India Indochina South of China	from 12th

The simultaneous acquisition of emission and excitation spectra facilitates a more accurate identification of dyes and lake pigments [14]. To maximize the extraction of the information present in these signals, this work proposes a chemometrics approach for the study of the database build up with historically accurate reproductions of brazilwood, cochineal, lac dye, and more recently kermes. These lake pigments were used to produce a similar range of colors, and the three anthraquinone based chromophores display similar excited state properties, **Figure 2.1**. The potential of chemometric models which simplify the interpretation of each system, i.e. each colorant, will allow to explore similarities between colorants and classify the spectral data into different classes. For this reason, hierarchical cluster analysis (HCA) and principal component analysis (PCA), as well as soft independent modelling of class analogy (SIMCA), were explored, with the spectral data acquired, to test the possibility of discrimination between these main four colorants.

PCA is the chemometrics workhorse. Its application is often intended to help interpretation of multivariate datasets. PCA projects multivariate data onto a lower dimension orthogonal space. These projections (loadings) yields the scores or an alternative representation of the samples, though encompassing most of the original data variance [27]. PCA is an unsupervised method in the sense that no considerations are made regarding the samples for building the model. HCA is the general designation of methods for grouping samples characterized by data vectors or matrices, eventually forming clusters. The distance between samples (e.g. Euclidean or Mahalanobis distance) is evaluated recursively, aiming at defining a clustering tree. With this grouping process, performed hierarchically, and depending on the selected algorithm, multiple clustering options are possible. Results are typically represented graphically in the form of a dendrogram, where samples are visualized according to their similarity [27]. The SIMCA model, is a supervised classification method. It is based on the development of multiple PCA models, each built considering samples of a known class or group [28, 29]. The goal is to allow for classification by presenting unknown samples to the different PCA models composing the SIMCA model. When projecting samples to this model, they are classified according to their similarity with the different PCA class models (typically Hotelling's T^2 and squared residuals statistics are used to evaluate the distance to each model). Indeed, when projecting one sample, different outcomes are possible: (1) the sample might be classified according to one class; (2) the sample is classified as belonging to two or more classes; (3) the sample is not classified in any of the model's classes. This allows for the coverage of high class variability by the principal components calculated individually, making SIMCA one of the most commonly used techniques for the classification of spectral data [28, 29].

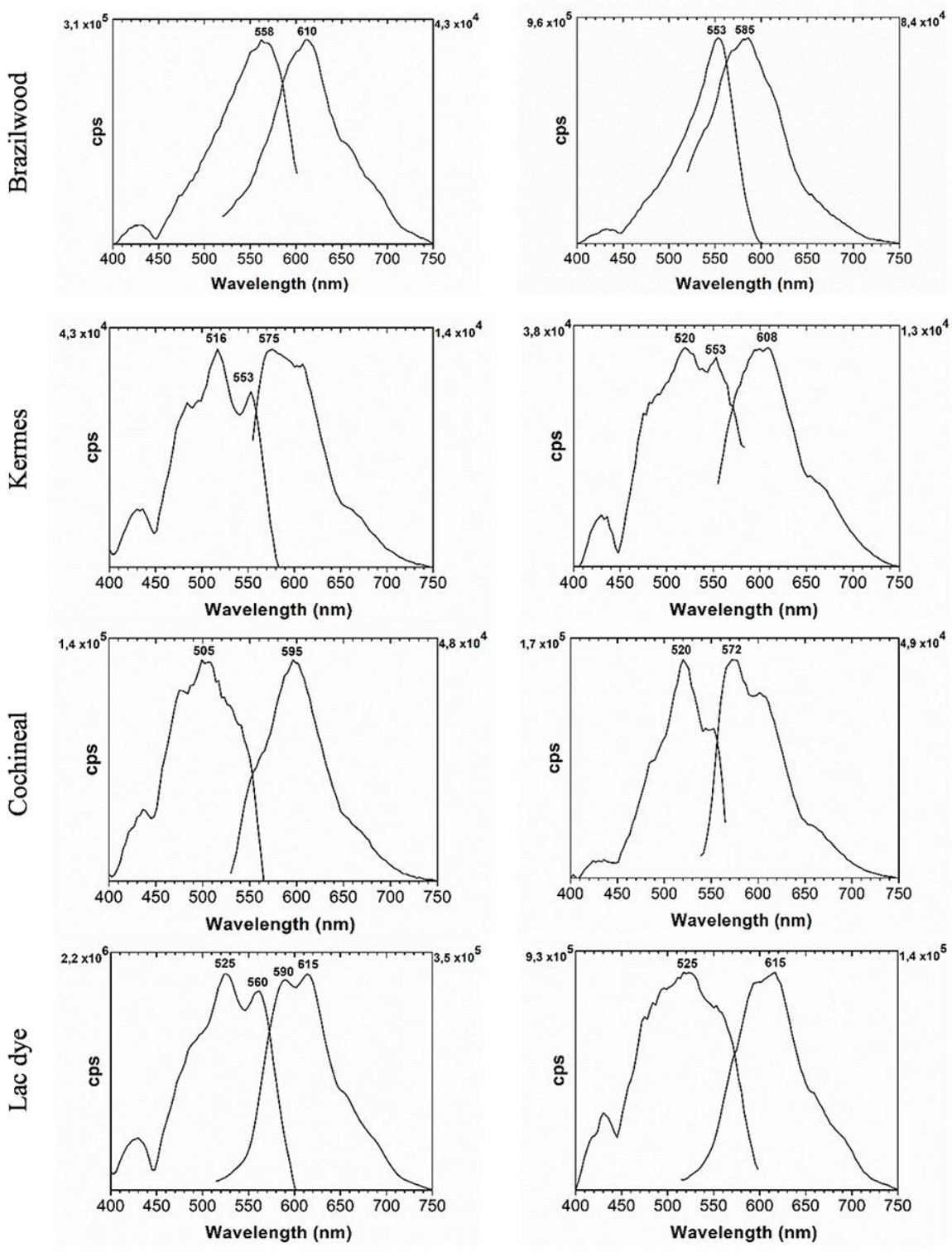


Figure 2.1. Excitation and emission spectra of selected reconstructions of the red lake pigments. From left to right: brazilwood, from the *Livro de como se fazem as cores*: recipe 8 and recipe 44; kermes, both from the Roosen-Runge adaptation of the *Jean le Begue* manuscript; cochineal, Winsor and Newton's *Finest Orient Carmine* and *Crimson with gypsum*; lac dye, *Ms. Bolognese*, recipe 129 and recipe B.140.

2.2. Methodology

2.2.1. Experimental

Historically accurate reconstructions

Kermes lake reconstructions were prepared, with as much historical accuracy as possible, according to the Roosen-Runge adaptation (1967) of a Jean le Begue´s manuscript (*Experimenta de coloribus*) recipe [30, 31]. *Kermes vermilio* female insects were ground in a mortar with additions of lye until a concentrated dark red solution was obtained. The mixture was heated for 30 min at 50 °C and then centrifuged for 10 min (pH circa 8). Afterwards, the dark red supernatant was heated at 50 °C and alum (Al^{3+}) was added (pH = 6.8). This procedure was repeated to verify the reproducibility of the data.

For lac dye, twelve recipes were selected from eight treatises/ recipe books: *Ibn Bādīs* (c. 1025), *Mappae clavicula* (9th – 12th c.), *Livro de como se fazem as cores* ('The book of all color paints', 15th c.), the *Jean le Begue manuscript* (1431), the *Bolognese manuscript* (15th c.), the *Strasbourg manuscript* (15th century), the *Montpellier manuscript* (15th c.) and the *Paduan manuscript* (late 16th to 17th c.); these reproductions have been described elsewhere [20, 31–36].

The production of cochineal lake pigments is very little documented in the written sources from the medieval period. Reconstructions of these lake pigments were therefore adapted from Winsor & Newton 19th c. archive in different varieties: carmine (*Finest Orient Carmine, Half Orient Carmine* and *Ruby Carmine*), and *crimson* (with an aluminate composed of alum and an alkaline compound, designated as *Crimson 1* and *2*, and with gypsum, designated as *Crimson with gypsum*). The preparation of the cochineal reconstructions is described in [22] and brazilwood reconstructions have been reported elsewhere [18].

Paint references were prepared using arabic-gum and glair. Glair was prepared as described on the 11th century *De clarea* treatise [37] and gum arabic, from Kremer Pigmente, was prepared according to *De arte illuminandi* as a 10% solution [38]. For the glair, the egg white was beaten and the liquid that formed at the bottom was used; for the gum arabic, the pieces were ground and then added to pure water. The lake pigments were first ground in an agate mortar with pure water and then ground with the binder. The paints were applied on filter paper and parchment with a paintbrush and allowed to dry. Spectroscopic or equivalent grade solvents and Millipore filtered water were used for all the spectroscopic studies as well as for the extraction of the dyes and preparation of the lake pigments.

Microspectrofluorimetry measurements

Fluorescence excitation and emission spectra were recorded with a Jobin–Yvon/Horiba SPEX Fluorog 3-2.2 spectrofluorometer hyphenated to an Olympus BX51M confocal microscope, with spatial resolution controlled by a multiple-pinhole turret, corresponding to a minimum 2 μm and maximum 60 μm spot, with 50 × objective. Beam-splitting is obtained with standard dichroic filters mounted at 45°; they are located in a two-place filter holder. For a dichroic filter of 570 nm, excitation may be carried out until about 560 nm and emission collected after about 580 nm ("excite bellow, collect above"). The optimization of the signal was performed daily for all pinhole apertures through mirror alignment, following the manufacturer's instructions, using a rhodamine standard (or other adequate reference). For the study of red dyes, two filter holders with two sets of dichroic filters are employed, 500 and 570 nm in one set and 525 and 600 nm in the other set. This enables both the emission and excitation spectra to be collected with the same filter holder. A continuous 450 W xenon lamp, providing an intense broad spectrum from the UV to near-IR, is directed into a double-grating monochromator, and spectra are collected after focusing on the sample (eye view) followed by signal intensity optimization (detector reading). The pinhole aperture that controls the area of analysis is selected based on the signal-to-noise ratio. For weak to medium emitters, it is set to 8 μm, in this work for very weak signals 30 μm spot was also used (pinholes 5 and 8, respectively) with the following slits set: emission slits = 3/3/3 mm and excitation slits = 5/3/0.8 mm. Emission and excitation spectra were acquired on the same spot whenever possible. For more details on the experimental set-up please see [13, 14].

The paint reconstructions were analyzed in situ. For each of the prepared paintings 6–9 emission and excitation spectra were acquired, in different days, in different points, and the data was shown to be reproducible. Forty excitation spectra with the corresponding emission spectra, were obtained for brazilwood; 34 spectra for cochineal; 22 spectra for kermes; and 22 spectra for lac dye. Therefore, 118 excitation spectra in total, together with the corresponding emission spectra, were used.

2.2.2. Theory and calculation

Spectral pre-treatment

Both excitation and the emission spectra were used. For each sample, excitation and emission intensity were independently normalized to 1 (area) and then the two data blocks were merged (horizontal concatenation of the matrices). For the excitation spectral dataset used in this work it was considered that some filtering was necessary combined with the removal of baseline drifts. It was decided to adopt the Haar transform and 1st derivative (2nd order) were selected for this task. Normalization by area (1) is also typically used for the analysis of fluorescence area and was also considered and applied subsequently to the first two methods [39]. See **Appendix A.1.1** for the spectral pre-treatment data.

Chemometrics methods

The HCA algorithm was made resourcing to the scores of PCA models. The number of principal components to use for the HCA was defined as the most appropriate for achieving a colorant class separation considering only the excitation dataset. Dendrograms considering from 1 to 10 components were tested. Colorants classification with the SIMCA method resourced on the development of models from a calibration dataset. Samples assignment to classes was defined according to a distance to model metric as described in [40]. The distance to model (d) as defined in **Equation 2.1** was used with the threshold 1.5 as the criterium for assigning samples to colorant classes.

Equation 2.1

$$d = \sqrt{\left(\frac{T^2}{T^2_{\mathrm{LIM},95\%}}\right)^2 + \left(\frac{Q}{Q_{\mathrm{LIM},95\%}}\right)^2} < 1.5$$

In Eq. 1, T^2 and Q are the Hotelling's T^2 and squared residuals statistics, respectively and $T^2_{LIM,95\%}$ and $Q_{LIM,95\%}$ are the confidence limits for a significance of 0.05. A sample is considered to belong to a class when d < 1.5. Prior to the application of all chemometric methods the data were mean centred. All chemometric analysis and data manipulations were performed in Matlab Version 8.6 (R2015b) (The Mathworks, Natick, MA) and the PLS Toolbox Version 8.2.1 (Eigenvector Research, Manson, WA).

2.3. Results and discussion

2.3.1. Historically accurate reconstructions

Brazilwood lakes and lac dye reconstructions encompass a chronological arch from the 15th c. until the 19th c. and 12th c. until the 16th c., respectively, also showing that the main steps for the manufacture of these lake pigments were kept through time [18, 32, 33]. Due to the lack of cochineal recipes in medieval records, 19th c. W&N carmine and crimson cochineal pigments were used [22]. The main process of W&N for carmine manufacture (Finest Orient Carmine) involved an acid extraction of the dye and the addition of aluminum from alum to calcium from milk. Two other processes to produce carmines involved the absence of the milk in the previous process (Half Orient Carmine) and extraction of the dye with potassium carbonate followed by precipitation with alum and cream of tartar (Ruby Carmine). The crimson colors were produced with the addition of a lake pigment dispersion to an aluminate composed of alum and an alkaline compound (ammonium or sodium carbonate), or an extender (gypsum) [22]. Kermes database is constituted of several reconstructions of a Jean le Begue's recipe [30, 31]. Further studies in other recipes are currently in progress.

Pigment lakes have been fully characterized and rationalized by multi-analytical techniques [18, 20, 22]. Analyzing the relative fluorescence intensities between colorants, we may conclude that brazilwood and cochineal present relatively similar excitation and emission intensities, while lac dye' intensity increases,

in some cases, by tenfold (see **Appendix A1.1**). Kermes, on the other hand, is the chromophore which presents the lowest intensities from the four colorants. The chromophores of both laccaic acid and carminic acid have been characterized in solution [41]. The quantum yield of fluorescence registered for these chromophores with a 1:100 ratio aluminum complex (lake), has been 1,5 x 10^{-2} and 4 x 10^{-2}, respectively, which enables their characterization as moderate and weak emitters. No values for brazilein-Al^{3+} complexes are available. However, knowing that brazilein at pH = 1.5 shows a quantum yield of fluorescence of 6.8 x 10^{-3} [42], we may predict a tenfold increase, for an aluminum complex, of the quantum yield, i.e., 7 x 10^{-2} [41]. A photophysical characterization is yet to be done on kermesic acid, which could shed some light on why the intensities are much lower than the other chromophores.

2.3.2. Unsupervised modelling

The pre-processed spectral data were analyzed by PCA. The first principal components were examined regarding the ability for separating samples with different colorants. The first and second components separate mainly brazilwood and cochineal from the other two colorants (lac dye and kermes), **Figure 2.2**. The third component differentiates the crimson recipes based on cochineal, where an aluminate or extender was added to a lake pigment dispersion, and the finest orient carmine colors, that stand out for the addition of milk as a source of calcium, **Figure 2.3**.

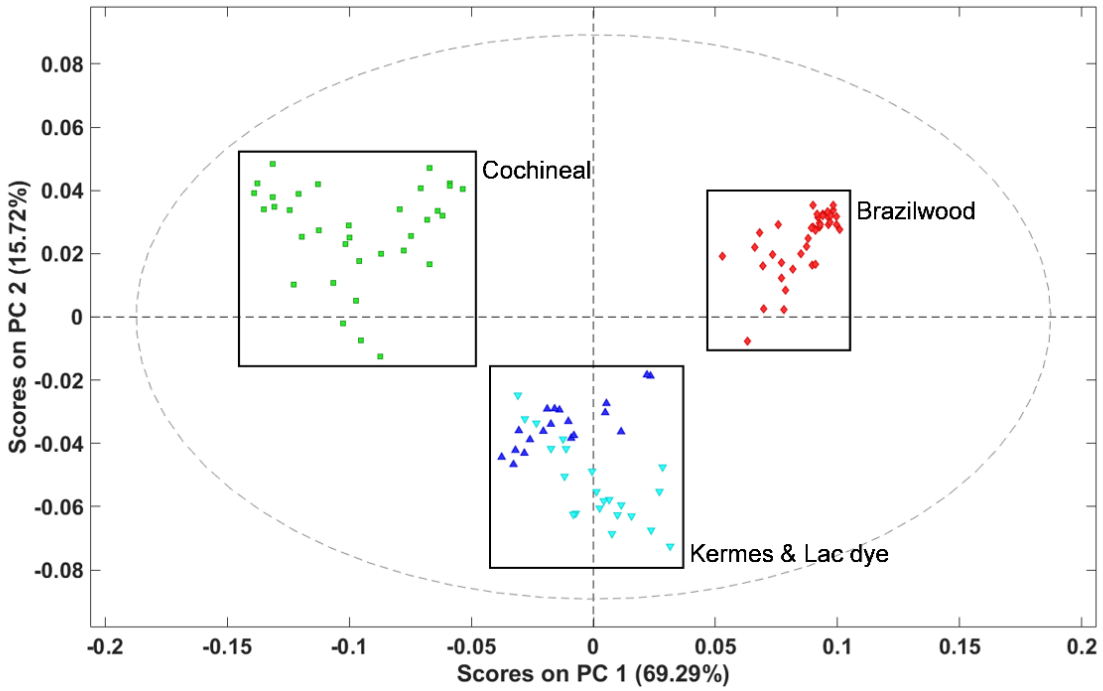


Figure 2.2. Principal component analysis scores, for normalized and filtered (by Haar transform and 1st derivative (2nd order)) excitation spectra for red lake pigments, showing the separation of cochineal (green) and brazilwood (red) from the other two colorants.

Even more interesting is the fact that it distinguishes between Crimson 1, which had the addition of an aluminate composed of ammonium carbonate and alum, and Crimson 2, which had the addition of

sodium carbonate and alum. This proves the potential of this methodology for the identification of not only the colorant, but also the specific recipe. The forth component separates the classes of kermes and lac dye, considering mostly the 400–440 nm region, **Figure 2.4**.

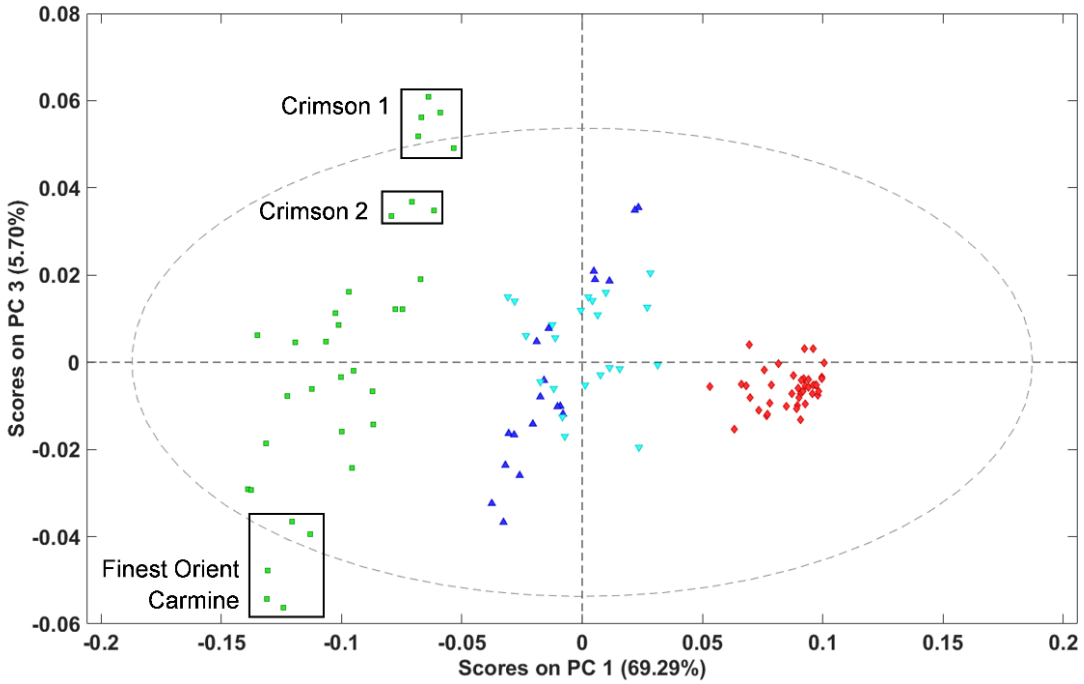


Figure 2.3. Principal component analysis scores illustrating the separation of cochineal manufacturing processes, Crimson lakes and Finest Orient Carmine (green).

The PCA model is not a classification method and, for a better visualization of the similarity between the different samples, the HCA method resourcing to the Ward's algorithm was used (using the Mahalanobis distance). The HCA method was fed with the principal components generated by PCA models. The number of principal components (PCs) to use was based on the development of different HCA considering different number of PCs but using only approximately 2/3 of the total samples (the dataset division was based on the Kennard-Stone algorithm). This was performed for emission/excitation spectra models. It was found that five components yielded the best colorants discrimination.

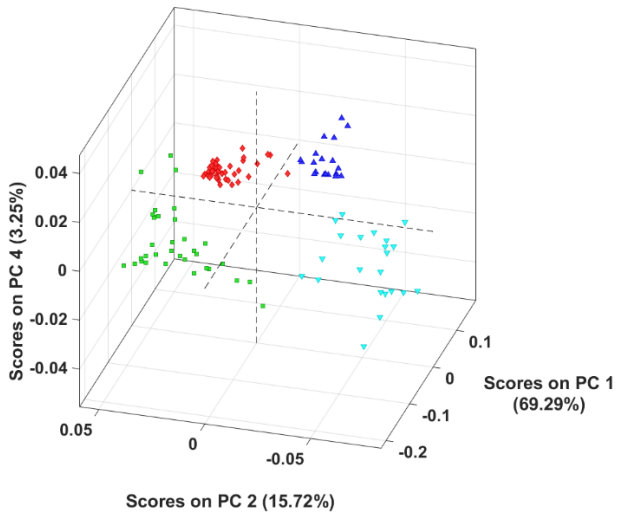


Figure 2.4. Principal component analysis scores, for normalized and filtered (by Haar transform and 1st derivative (2nd order)) excitation spectra for red lake pigments, illustrating the separation of the red lake pigments: kermes (light-blue), lac dye (dark-blue), cochineal (green) and brazilwood (red).

After this selection, the HCA method was applied to all samples considering always five components. These results are presented subsequently. Considering the excitation spectra dataset alone, the HCA method revealed a successful separation of the four dyes, **Figure 2.5**. Four distinct clusters are visible in the dendrogram, each encompassing the samples of a different colorant. When both excitation and emission spectra sets were used, the distinction between lac dye and kermes was not possible due to the similarities in the emission spectra for these colorants, which is also seen in **Figure 2.2**.

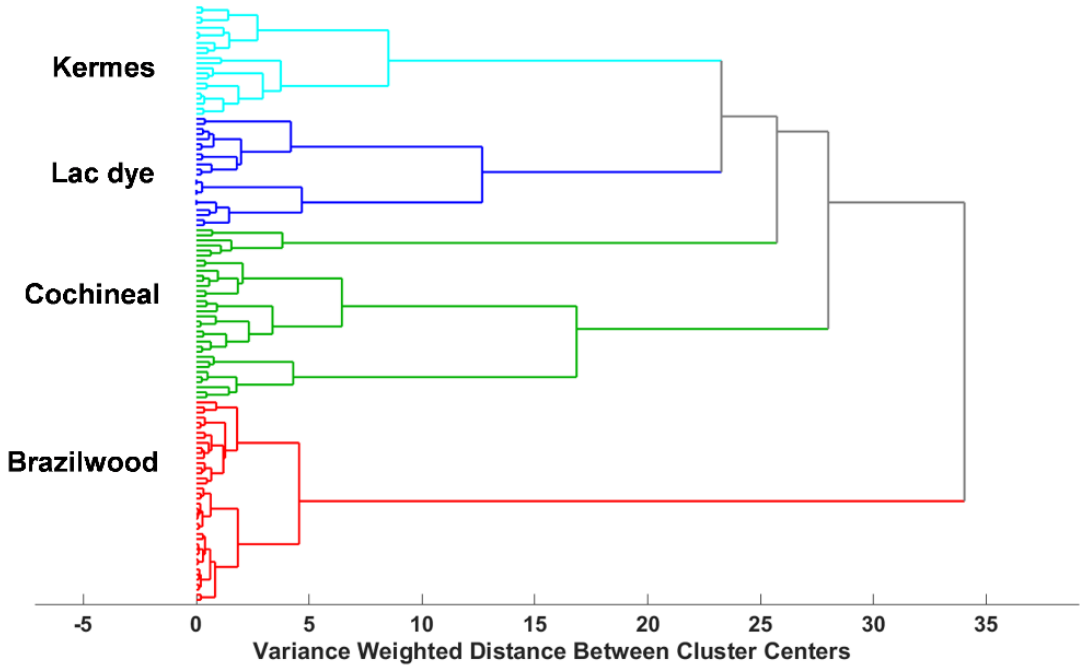


Figure 2.5. Dendrogram generated by HCA applied to excitation spectra, showing a clear discrimination between the four red lake pigments: kermes (light-blue), lac dye (dark-blue), cochineal (green) and brazilwood (red).

2.3.3. SIMCA model

As previously mentioned, the SIMCA model, is a supervised classification method, allowing the development of multiple PCA models, each built considering samples of a known class or group. To build the SIMCA method, samples were divided randomly in a training and validation set according to the Kennard-Stone algorithm, where 2/3 of the 118 samples were set for calibration and the remaining for validating the model. Four PCA models were calibrated considering the excitation and emission spectra of the four colorants. The criteria for selecting the number of components was based on the percentage of captured variance (at least 95%). Brazilwood, cochineal and lac dye samples were modelled with four components, while kermes demanded five components. Optimized PCA models were then built using the entire calibration dataset. Each model was then tested by projecting the validation samples. In SIMCA, the normalization by area proved to be enough as a pre-treatment.

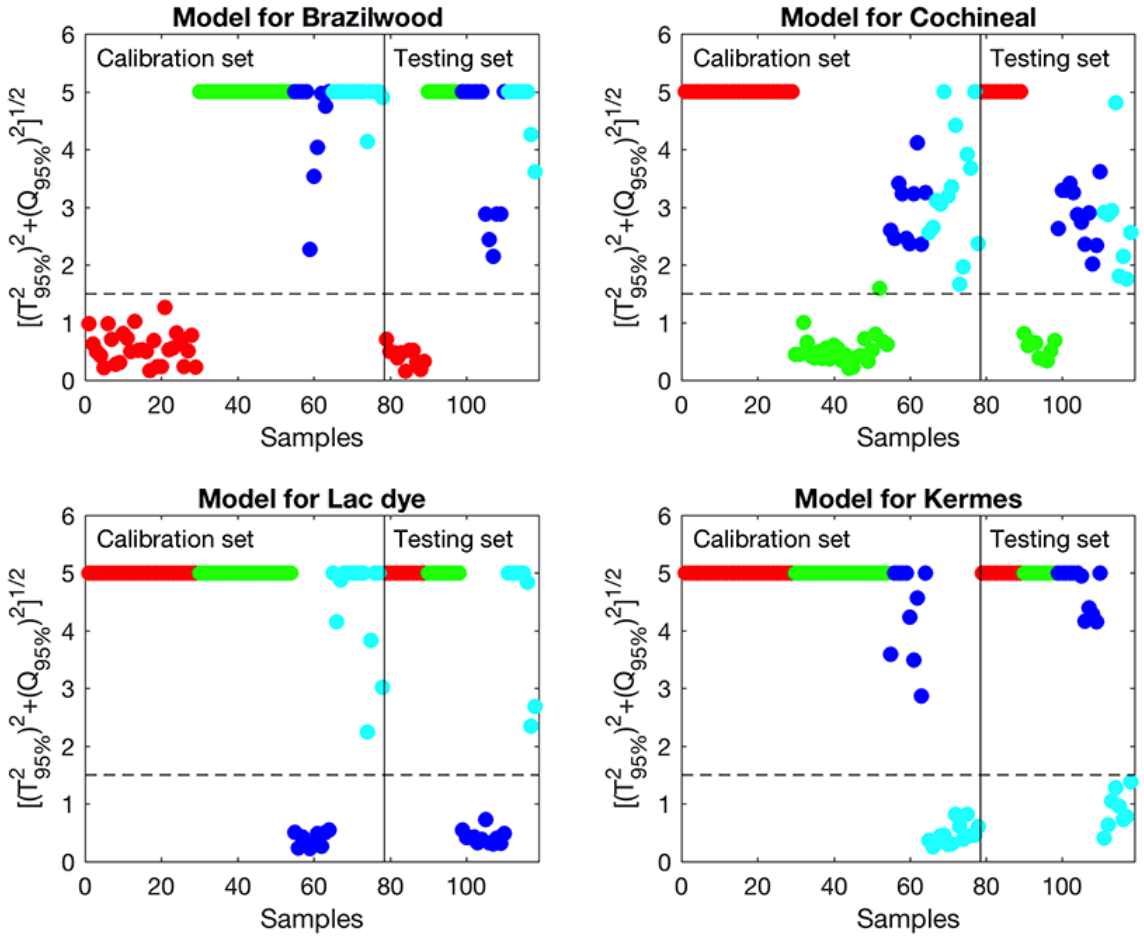


Figure 2.6. Distance to model metric used to assign samples (calibration and test sets) to colorant classes resulting from the SIMCA modelling approach: kermes (light-blue), lac dye (dark-blue), cochineal (green) and brazilwood (red). The SIMCA modelling approach results in 100% correct predictions for both calibration and validation sets.

Both training samples and validation samples were predicted as the correct class as shown in **Figure 2.6**. The distance to model for each sample (Calibration and Testing sample) for the four different PCA models forming the SIMCA approach are presented in **Figure 2.6** together with the threshold level for class assignment (1.5). For a better visualization of the data, each colorant is represented with a specific color. Also, distance to model values were truncated to five for better results visualization. Clearly, brazilwood samples (red markers on **Figure 2.6** top-left) lie below the 1.5 threshold meaning that these samples are close to the brazilwood model as expected. Other samples lie significantly above the 1.5 threshold. This result can be observed for all colorant models. There are no samples that could belong to more than one class. The strict application of the colorant assignment criterium results on 100% of correct classifications for all validation samples. It was demonstrated the ability of the SIMCA modelling approach to correctly assign the colorant type to all validation samples resourcing to the excitation and emission spectra.

2.4. Conclusions

Microspectrofluorimetry is a powerful technique for the analysis of dyes and lake pigments, due to the advantage of being used in situ without any contact with the sample or work of art to be analyzed. In this work, this technique was explored in a robust methodology for the identification of red lake pigments, using a chemometric approach that allowed to explore similarities between colorants and classify the spectral data into the four different colorant classes, **Figure 2.1**. Unsupervised (HCA & PCA) and supervised (SIMCA) modelling were tested in the discrimination between the four dye families. The first was applied considering the excitation spectral dataset alone and with several pre-processing treatments, allowing for the separation of the colorants into different clusters. It was also possible to pin point the main W&N's manufacturing processes of cochineal lake pigments, where among the crimson lakes it was possible to distinguish between the different additives, aluminates (Crimson 1 and 2) and the gypsum, and among the carmine colors between the Finest Orient Carmine, which had the addition of milk as a source of calcium, and the Half Orient Carmine and Ruby Carmine, both without calcium.

The SIMCA modelling, allowed for the discrimination between chromophores, with both spectral sets, i.e. excitation and emission, while using fewer pre-processing treatments. Based on these results, this methodology will be next applied in data acquired form artworks, from medieval manuscripts to textiles, to select which modelling (unsupervised or supervised) best suits the data. Finally, a search algorithm will be developed making this new advanced analytical tool accessible to the conservation community, and not only to the photophysics experts.

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Organic colorants as markers for a chronology and geography of medieval *scriptoria* and workshops: a chemometrics approach.
Forthcoming.

Chapter 3. Organic colorants as markers for a chronology and geography of medieval *scriptoria* and workshops: a chemometrics approach

Abstract

Historical paints based on lac dye and brazilwood lake pigments were tested as putative markers for a chronology and place of production of medieval illuminated manuscripts. These paints had been previously fully characterized through a multi-analytical approach. In this work, molecular fluorescence spectra were acquired and analysed from manuscripts dating from 12th c. into 15th c. that were produced in monastic scriptoria and lay workshops. First, the shapes and intensity of the emission and excitation spectra were discussed in detail by comparison with reference compounds (produced at different pH, different alum concentration, different fillers). Based on this detailed analysis, the spectra were assembled in groups that correlate with recipe specificities. Second, statistical modelling was applied to the same fluorescence spectra and the grouping generated was compared with the previous. Except for some misplaced spectra, most of the groups were confirmed. The misplaced paints were the result of either a poor signal to noise ratio or occurred because certain paints were so dissimilar that they could not be grouped. For the first time, we could pinpoint a formulation in which lac dye and brazilwood chromophores are admixed, in manuscripts from the Alcobaça scriptorium (De avibus and Sermones de verbis Domini, Alc 238 and 347, respectively). This could not be previously observed, neither in the infrared nor the Raman spectra (in which only the presence of shellac could be detected). These paints presented a very good match with a recipe that we reproduced from the text of Jean Le Begue. This allow us to hypothesize that, with further developments, molecular fluorescence could be used as a tool to provide geographic information on the place of the manufacture of an illuminated manuscript as well as on its dating.

3.1. Introduction

The understanding and prevention of light-induced color changes in artworks depends on the chemical comprehension of the excited-state behaviour of pigments and dyes in relation with the matrix and the environmental surrounding [1]. Our research has shown that microspectrofluorimetry is a fundamental tool to identify organic dyes in complex matrixes, being essential to the study of their photophysics and the understanding of their photochemistry [1-5].

Building on our previous work on the applications of microspectrofluorimetry in heritage science, we aim to develop a search function that will allow the identification of organic colorants in medieval artworks [5-9]. Based on the specificities of the paint formulation (the original recipe and its making), it also aims in providing details on the conservation conditions and site of production. The algorithm will compare excitation and emission spectra of a reference database with unknowns. As a first approach to the testing of the search algorithm, we selected medieval manuscript illuminations, because they provide dye diversity in an acceptable conservation condition. Our first reference database combined a set of medieval color reconstructions that included lake pigments and paints for the following natural colorants: kermes, lac dye, brazilwood, and cochineal [8]. The results obtained were very promising, and this prompted us to built-up and test a reference database that now combines spectral data acquired on medieval manuscript illuminations for lac dye and brazilwood chromophores, **Figure 3.1** to **Figure 3.3**. What motivate us to test a reference database based on original medieval colors? and why lac dye and brazilwood chromophores? Using original medieval colors will allow us to probe the medieval know-how on paint making and its specificities, contributing to a better understanding of how creative processes were developed in medieval workshops and *scriptoria*, and to know more on the makers of these artworks. Depending on the overall paint formulation (pigments, dyes, binding media and other additives, e.g., as fillers, and their relative proportions), a same chromophore may display different spectral shapes and intensities. The use of a medieval database will pave the way to research on identification markers, but it presents as disadvantage the fact that we do not control the conservation condition of our paint (the history of the object that will also be reflected on the spectra obtained). To be able to assess the conservation condition, with a satisfactory level, we selected two chromophores that we have extensively studied, lac dye and brazilwood [4, 9-14]. They also represent the most important reds/pinks/purplish colors used in medieval illuminations [15, 16]. The intrinsic complexity of lac dye paints and the variety of brazilwood lake pigment formulations will prompt us to be careful when discussing if a certain spectral change is due to a certain formulation or is a consequence of a color change over time, in our case studies, eight to six centuries.

We consider this research work, as a first approach to tackle a formidable challenge: to identify and preserve organic colorants in works of art, in an efficient mode and providing in-depth knowledge. The research that we will present and discuss in this paper is a new starting point and, possibly, it will introduce more questions than it will provide answers. On the other hand, it will certainly feed a necessary trend in chemistry for heritage science, in which "*we can envision physicochemical research addressing the structural, electronic and reactive properties of cultural heritage materials nourished by phenomenological studies unravelling the rich information enclosed in a work of art*" [1].

Our reference database combines spectral information acquired in manuscripts preserved in Portuguese collections, from Portuguese, French and Flemish production, dating from the 12th c. into the 15th c., please see **Figure A1.14** from **Appendix A1.2**. The Portuguese production is essentially based on the monastic *scriptoria*, whereas Flemish and French in lay workshops, possibly active in flourishing medieval cities. Spectra were acquired both in situ and in microsamples (invisible to the naked eye). We tested both sets, and although we had a higher number of spectra acquired in situ, we

choose to present in this work the data acquired in micro-samples, because we could guarantee a higher reproducibility.

Our modus operandi will be as follows. We will start by grouping our data by correlating the shape and intensity of the spectra with reference samples that were prepared with different pH, different Al^{3+} concentrations, different fillers, etc. Afterwards we will apply statistical functions and compare the results with our previous grouping. We will summarize whenever possible our main results at the end or when initiating a session, providing the relevant information to the non-expert in photophysics. The chemometrics grouping will be discussed and correlated with the paint formulation previously characterized through a multi-analytical approach [11, 12, 17-32].

The statistical methods employed in this work have the objective of keeping the systematic profiles captured by the analytical methodologies thus unveiling consistent patterns that help us to differentiate, cluster and therefore perform a systematic analysis of the collected data. As fluorescent signals may suffer the influence of other factors other than those relevant to this work, it is the purpose of the selected methods to, as most as possible, provide a reliable and robust analysis of the signals without these potential interferences that goes from different signal-to-noise ratio to the existence of some chemical compounds that may affect the microspectrofluorimetry signals' shape. The methods resourced to accomplish the analysis task were successfully used in the conservation area. In this work, hierarchical cluster analysis and principal component analysis were used. Examples of the applications of these methods in related works can be found elsewhere [8].

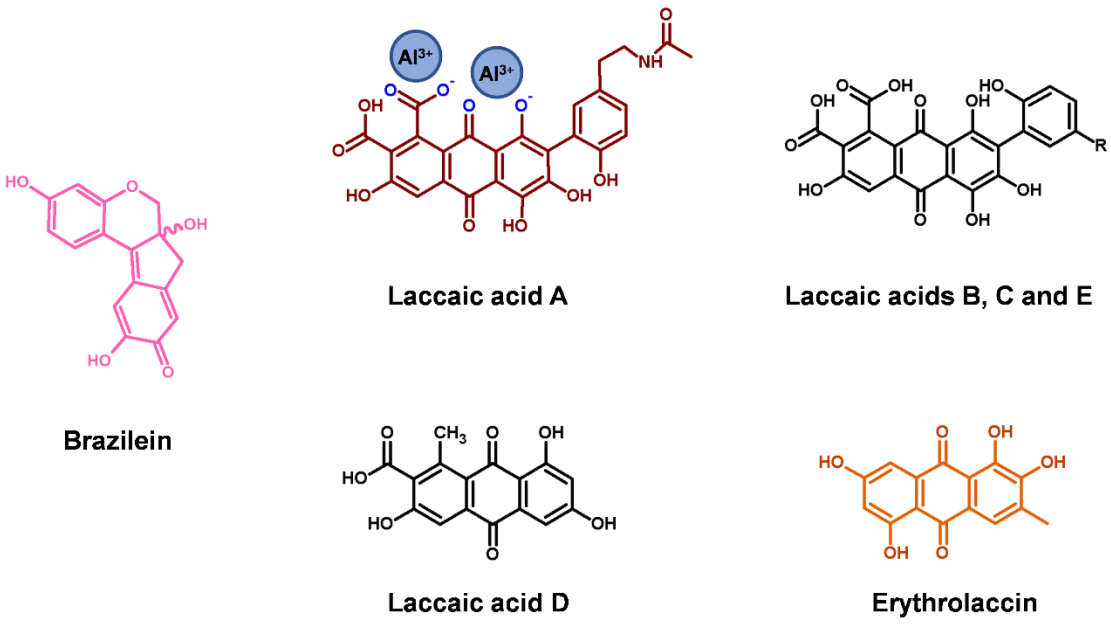

Figure 3.1. Chemical structures of brazilein, laccaic acid A, laccaic acid B, C and E (B, R=CH_2CH_2OH; C, R=CH_2CHNH_2COOH; E, R=$CH_2CH_2NH_2$), laccaic acid D and erythrolaccin. For laccaic acid A is shown the main sites for complexation with a metal ion.

Figure 3.2. Lac dye details from manuscripts showing the differences in hues of original paints: from left to right, lac dye in Lorvão 15 fol. 26, Santa Cruz 1 fol. 21v and Santa Cruz 20 fol. 191. The figures below are macro details of the top row figures.

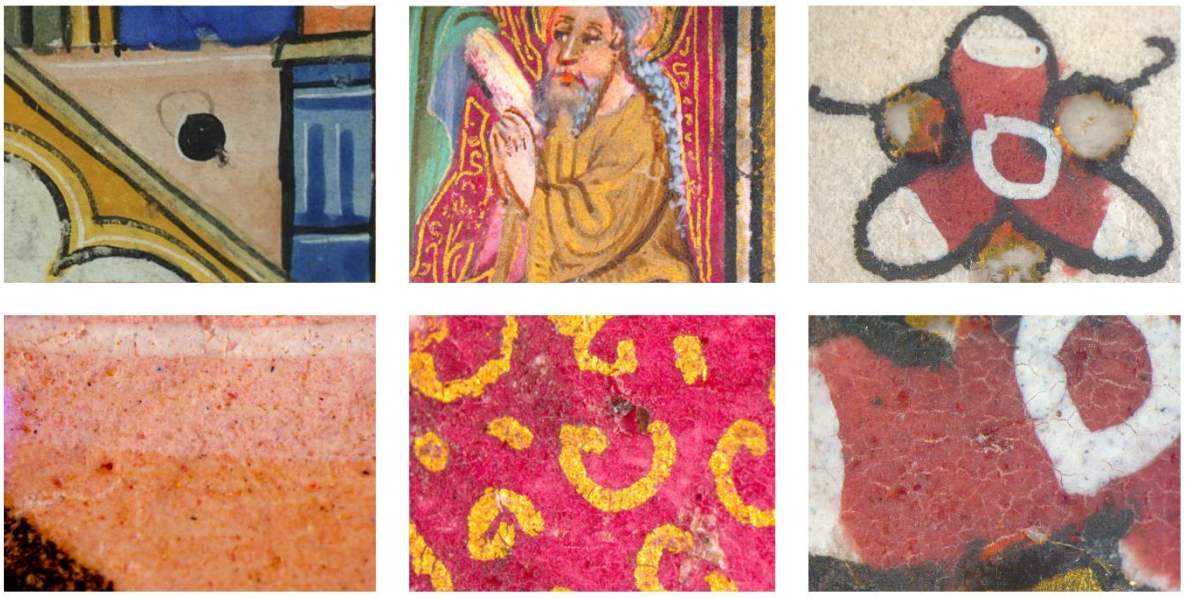

Figure 3.3. Brazilwood details from manuscripts showing the differences in hues of original paints: from left to right, Ajuda Songbook fol. 59, IL 15 fol. 66 and Cofre nº 22 fol. 76v. The figures below are macro details of the top row figures.

3.2. Experimental

3.2.1. Artworks

Seventeen manuscripts from the 12th to the 15th century from *scriptoria* of the three main Portuguese monasteries, São Mamede of Lorvão, Santa Cruz of Coimbra and Santa Maria of Alcobaça, were selected. Lorvão 5 (1183-4), 12 (13th c.), 13 (13th c.), 15 (1201-50) and 50 (1183), from São Mamede of Lorvão, now at BPMP; SC 1 (1151-1200), SC 20 (early 13th c.) and SC 21 (early 13th c.), from Santa Cruz of Coimbra, at ANTT; Alc. 238 (late 12th c.), 247 (12th c.), 249 (13th c.), 347 (12th – 13th c.), 412 (1257), 419 (12th – 13th c.), 421 (12th – 13th c.), 427 (12th – 13th c.) and 446 (13th c.), from Santa Maria of Alcobaça, now at BNP [11, 17-24]. These have been previously analysed by Ana Claro, Catarina Miguel and Rita Castro [11, 17-24].

The Ajuda Songbook (Palace of Ajuda Library, 13th – 14th c.), Alc. 54 (BNP, 14th – 15th c.) and six Books of Hours, cofre nº 22 (PNMAF, 1400-20), cofre nº 24 (PNMAF, 1420 / 1470), IL 15 (BNP, ca. 1450), IL 19 (BNP, 1420-30), IL 21 (BNP, 1460-70) and IL 42 (BNP, ca. 1470), were equally selected for this study [12, 25-32]. The data were originally acquired by Paula Nabais, Tatiana Vitorino, Rita Araújo and Rita Castro [12, 25-32], however, the microsamples available were reanalysed by Paula Nabais for this work with a different dichroic filter set. See below for more information.

The data used in this study are provided in **Table A1.5** - **Table A1.9** and **Table A1.13** - **Table A1.15** in the **Appendix A.1.2**. Comparison with cochineal is also in **Appendix A.1.2**.

3.2.2. Microsampling

Micro-sampling of the manuscripts was performed with a microchisel from Ted Pella microtools under a Leica KL 1500 LCD microscope (7.1x to 115x objective) and a Leica Digilux digital camera, with external illumination via optical fibers. Micro-samples were taken under a microscope, typically of 20–50 μm in diameter and as such invisible to the naked eye; as we have not yet obtained their weight, even though micro-scales have been used, we can use its detection limit to conclude that they weigh less than 0.1 μg.

3.2.3. Microspectrofluorimetry measurements

Fluorescence excitation and emission spectra were recorded with a Jobin–Yvon/Horiba SPEX Fluorog 3-2.2 spectrofluorometer hyphenated to an Olympus BX51M confocal microscope, with spatial resolution controlled by a multiple-pinhole turret, corresponding to a minimum 2 μm and maximum 60 μm spot, with 50x objective. Beam-splitting is obtained with standard dichroic filters mounted at 45°; they are located in a two-place filter holder. For a dichroic filter of 570 nm, excitation may be carried out until about 560 nm and emission collected after about 580 nm (“excite bellow, collect above”). The optimization of the signal was performed daily for all pinhole apertures through mirror alignment, following the manufacturer’s instructions, using a rhodamine standard (or other adequate reference).

For the study of red dyes, two filter holders with two sets of dichroic filters are employed, for lac dye the set of 500 and 600 nm and for brazilwood the set of 540 and 600 nm. This enables both the emission and excitation spectra to be collected with the same filter holder. A continuous 450 W xenon lamp, providing an intense broad spectrum from the UV to near-IR, is directed into a double-grating monochromator, and spectra are collected after focusing on the sample (eye view) followed by signal intensity optimization (detector reading). The pinhole aperture that controls the area of analysis is selected based on the signal-to-noise ratio. For weak to medium emitters, it is set to 8 μm, in this work for very weak signals 30 μm spot was also used (pinholes 5 and 8, respectively) with the following slits set:emission slits = 3/3/3 mm and excitation slits = 5/3/0.8 mm. Emission and excitation spectra were acquired on the same spot whenever possible. For more details on the experimental set-up please see [5, 6].

3.2.4. Fourier transform infrared microspectroscopy

Infrared analyses were performed using a Nicolet Nexus spectrophotometer coupled to a Continuμm microscope (15 x objective) with a MCT-A detector cooled by liquid nitrogen. The spectra were collected in transmission mode, in 50 μm areas resolution setting 4 or 8 cm^{-1} and 128 scans, using a Thermo diamond anvil compression cell. For some infrared spectra the system was purged with nitrogen prior to the data acquisition; for all infrared spectra the CO_2 absorption at circa 2400-2300 cm^{-1} was removed from the acquired spectra (4000-650 cm^{-1}). To improve result robustness, more than one spectrum was acquired from different sample spots.

3.2.5. Data analysis

Spectral pre-treatments

Excitation and emission spectra were tested, separately and in combination. Each spectrum was pre-processed by normalization to unit area. Spectra of different samples were then arranged vertically forming two data blocks (excitation and emission) forming two-way matrices (samples versus wavelength). These data blocks were analysed in separate and in combination. In the latter situation, the two blocks were merged by horizontally concatenating the matrices. The best results were accomplished with the combination of the excitation and the emission spectra. Further details on the selection of pre-processing methods can be found in [34].

Chemometric methods

Hierarchical cluster analysis (HCA) was applied to the spectral data towards the discrimination and classification of artworks using different dye families. Dendrograms were developed considering data from the entire artworks' dataset including the whole wavelength range. HCA was applied on the first

three principal components and Euclidean distance. The Ward's algorithm was used to perform the clustering approach. Prior to the application of all chemometric methods, the datasets were mean centred. All chemometric analyses and data manipulations were performed with Matlab Version 8.6 (R2015b) (The Mathworks, Natick, MA) and the PLS Toolbox Version 8.2.1 (Eigenvector Research, Manson, WA).

3.3. Results and discussion

3.3.1. Lac dye in medieval manuscripts

Table 3.1. A total of 70 emission and 70 excitation spectra on 37 lac dye paints were collected from 17 illuminated manuscripts produced in Portuguese monasteries during 12th c.-13th c. Based on their spectral features these spectra were assembled in 4 groups.

		Group Lac 1	**Group Lac 2**	**Group Lac 3**	**Group Lac 4**
Excitation maxima		**473 nm** band at 430 nm	**535-555 nm**	**525 nm** *sh* 500 nm (resin chromophores) *sh* 550-555 nm	**554 nm**
Intensity	Max	4.2 x 10^5	4.8 x 10^5	6.5 x 10^5	1.0 x 10^6
	Min	1.3 x 10^5	2.6 x 10^5	2.2 x 10^5	4.6 x 10^5
Emission maxima		**588-593 nm** *sh* 562 nm *sh* 615 nm	**585-590 nm** *sh* 563 nm *sh* 610-616 nm	**585-590 nm** *sh* 610-616 nm	**600-610 nm** **592-594 nm** *sh* 610 nm
Intensity	Max	8.1 x 10^4	9.2 x 10^4	1.3 x 10^5	9.3 x 10^4
	Min	2.1 x 10^4	3.4 x 10^4	2.0 x 10^4	3.6 x 10^4

Fluorimetric and infrared analysis of lac dye paints

In a previous publication, based on medieval reproductions of lac dye paints, we proposed that lac dye reds were obtained as "free lac dyes" and Al^{3+}-lac complexes [9]. Fourteen recipes for lac dye were selected from 8 treatises / recipe books combining knowledge from the 10th century up to the late 16th century; four of these recipes did not employ an aluminium salt[24] [10]. "Free lac" paints are not complexed with Al^{3+} and display the following spectral features, as seen in ***group Lac 1***; *i)* a broad excitation spectrum with a maximum at 473 nm (and possibly also a band at 430 nm that appears in the

[24] Ms. of *Ibn Bādīs* (c.1025), Chapter 6; *Ms. The book of all color paints* (15th century), Chapter 13; *Ms. Paduan* (late 16th–17th century), Recipe 90 and 113 [10, 35-37].

same region in which we have an instrumental artefact of low intensity), **Figure 3.4** and **Table 3.1**; *ii)* an emission spectrum with a maximum at 588 nm and shoulders at 563 nm and 600 nm, **Figure 3.4**. From 8 original manuscripts (from a total of 17), 12 paints are included in this ***group Lac 1***[25], see **Table A1.5** from **Appendix A1.2**. Also, several reproductions of paints described in medieval written sources may be included in this group [10]. For example, the paints that were obtained reproducing the recipes described in the manuscript of *Ibn Bādīs* (c.1025), chapter 6; *The book of all color paints* (15th c.), chapter 13; and manuscript *Paduan* (late 16th -17th century), recipes 90 and 113 [10, 11, 35-40], **Figure 3.5**.

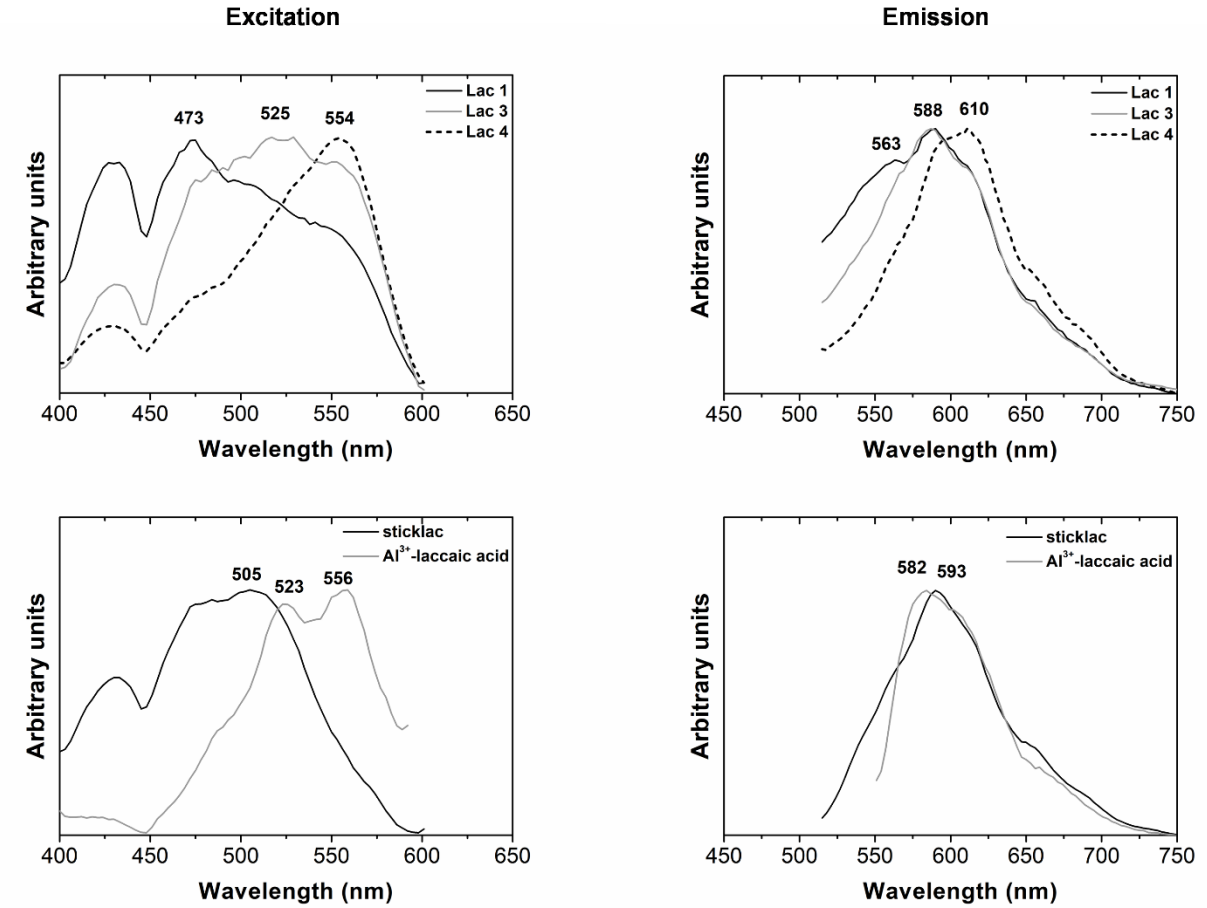

Figure 3.4. Lac dye paints excitation and emission spectra: of lac dye paints in 12th c.-13th c. Portuguese manuscripts that are represented as groups Lac 1 (—), Lac 3 (—) and Lac 4 (····); and of sticklac (raw material, resin + chromophores); Al^{3+}-laccaid acid A complex, applied on filter paper, pH=3.5. For more details please see text.

The excitation spectrum for Al^{3+}-lac complexes is different from the one of free lac dye, being characterized by the two bands at ca. 554 nm and 525 nm as well as a shoulder at ca 500 nm that is characteristic of sticklac, **Figure 3.4**, as seen in ***group Lac 3*** [9]. On the other hand, its emission spectra share, with free lac dye, a maximum at 588 nm, being distinguished by the absence of the shoulder at 563 nm, **Figure 3.4**. From 6 original manuscripts (from a total of 17), 7 paints are included in this ***group***

[25] ***Group 1*** - SC 20, fols. 191 and 197v; Lv 13, fols. 21, 30 and 44v; Alc 412, fols. 10v and 12; Alc 421, fol. 202v; Alc 446, fol. 96v, Lv 12, fols. 17 and 94; and SC 21, fol. 19.

Lac 3[26], see **Table A1.7** from **Appendix A1.2**. Also, several reproductions of paints described in medieval written sources may be included in this group [10]. For example, *Ms Mappae Clavicula* (12th century), recipe 253; *Ms Bolognese* (15th century), recipes 129, 131, 137 and 140; *Strasbourg manuscript* (15th century), recipe for Bright Paris Red; *Montpellier Ms* (15th century), recipe 1.9; and *Jean le Begue* (1431), recipe 36, **Figure 3.5** and **Figure 3.6** [9-11, 35-40]. The spectra from the reconstructions have, generally, a better signal-to-noise ratio, as seen in **Figure 3.6**, since they have not been through centuries-old aging. Most spectra obtained in the medieval manuscripts may be assembled in one of these two main groups, ***Lac 1*** and ***Lac 3***, or share certain features of one or both as follows, **Figure 3.4**.

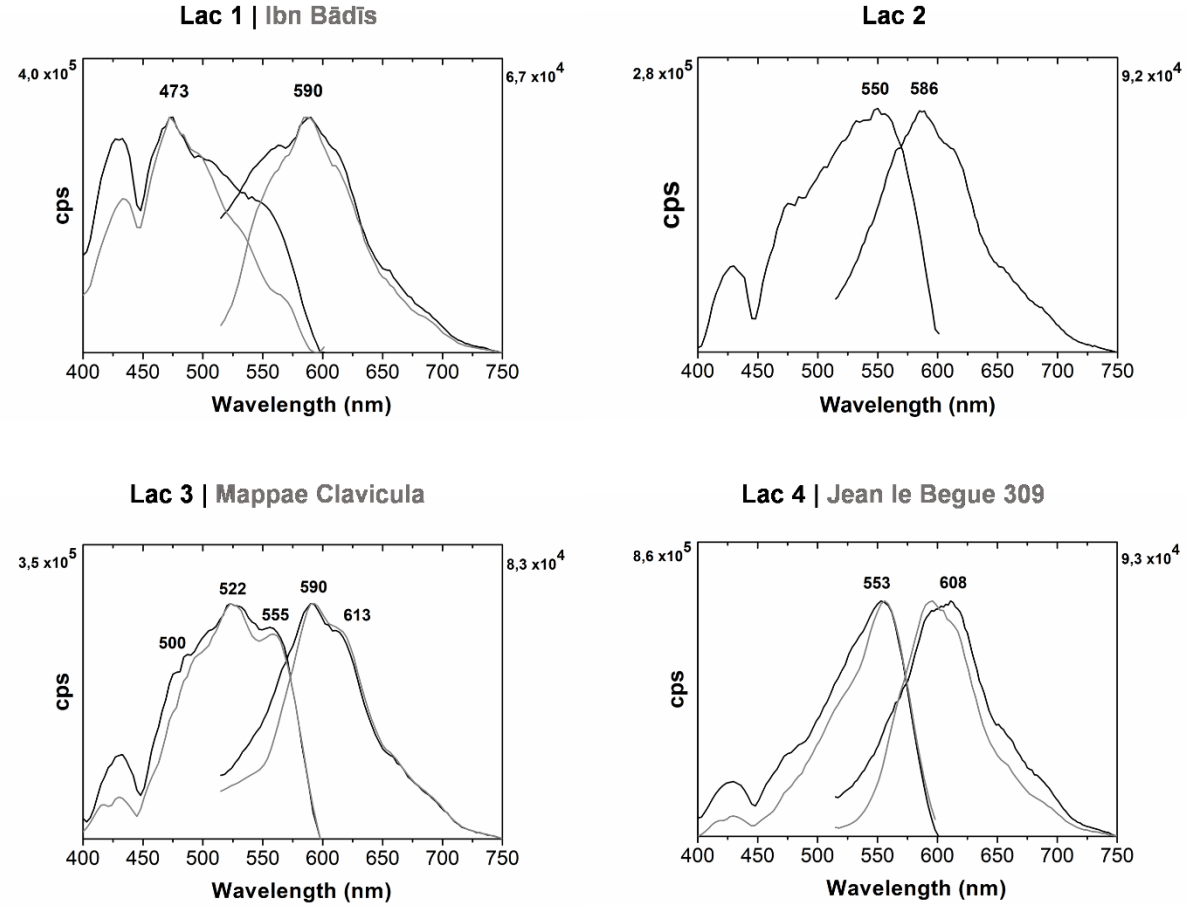

Figure 3.5. Lac dye paints excitation and emission representative spectra of each group compared with reference paints applied using glair as binder on filter paper; for group Lac 2 it was not possible to find a match with a medieval reconstruction. ***Group Lac 1***, Alc 412 fol.10v with an *Ibn Bādīs* reconstruction. ***Group Lac 3***, Alc 247 fol.21v with a *Mappae Clavicula* reconstruction. ***Group Lac 4***, Alc 238 fol.206v with a reconstruction from recipe 309 of the *Jean le Begue* Ms.

In ***group Lac 2*** (see **Table A1.6** from **Appendix A1.2**), the fluorescence emission spectra display the features representative for group Lac 1, but different excitation spectra, **Figure 3.5**. These excitation spectra are still characterized by a very broad band, but the maxima fall at longer wavelengths, between

[26] ***Group 3*** - Lv 5, fols. 6 and 73v; Lv 13, fol. 44v; Lv 15, fol. 26; SC 21, fol. 2; Alc 247, fol. 21v, and Alc 421, fol. 193v.

535-555 nm (corresponding more or less to the region in which the first band of the complex Al^{3+} -lacaic acid A emits) It resembles, to a certain degree, the excitation spectra that we obtain with brazilwood lake pigments (see **3.3.2**). From the original manuscripts 9 paints are included in ***group Lac 2***[27]. In this case, we could not find a medieval recipe that described a lac dye paint with these specific spectral features.

The fluorescence spectra from ***SC1 paints*** are possibly the more complex in the collection, being characterized by a distinctive set of spectra (fols. 2v, 37 and 77); the excitation spectra may be compared with that displayed by group Lac 2, but the emission spectra show a resolved envelope with two maxima at ca 560 and between 580-86 nm (like a spectra from group Lac 1, in which the shoulder has transformed into a band), see **Table A1.9**. We could not find a match in any of our historical reconstructions. Other two folios of the same manuscript present a distinct set of spectra: folio 14v can be compared with group Lac 1, with an excitation maximum at 474 nm and an emission maximum at 593 nm, while folio 24 presents the same excitation maxima as the latter, but with a shifted emission maxima, circa 560 nm with a shoulder at 580 nm, resembling the emission of folio 37.

Finally, spectra from ***group Lac 4*** (see **Table A1.8** from **Appendix A1.2**), were only found in four paints belonging to three folios of two manuscripts (Alc 238 and Alc 347), displaying different shapes and maxima shifted to higher wavelengths when compared to the other groups. In **Figure 3.5**, two representative spectra are depicted, being characterized by an excitation maximum at 554 nm and emission at ca 592-610 nm. In this case, it was possible to find a very good match with a lac dye paint reproduced following the instructions in recipe 309 from *De diversis coloribus* that is included in the manuscript compiled by *Jean Le Begue* (1431), which employs both lac dye and brazilwood [10, 36]. The excitation spectrum is what better distinguish this recipe from all the other medieval lac dye paints, and the spectral envelope of the original manuscripts compares very well with the *Le Begue* 309 recipe, both in shape and intensity. This paint combines spectral features from two different chromophores that are both medium to weak emitters, and depending on the raw materials, their quantities and processing as well as on the acquisition conditions, one may dominate over the other. As a matter of fact, in the fourteen recipes selected to be reproduced, two included a mixture of lac with brazilwood; in one of the recipes, the quantity of both materials is indicated[28] whereas in the second, *Le Begue* 309 recipe, only a vague description is given: "*Take an ounce of lake, and rasp finely a little Brazil wood*" [10, 36]. See **Table A1.10** from **Appendix A1.2** for the spectra of the historical reconstructions.

In all the medieval lac dye paints, SERS could prove the use of lac dye in its manufacture [9, 11], including in the two manuscripts above described (Alc 238 and Alc 347). However, we predict that it would be very difficult to detect brazilwood chromophore in a mixture with lac dye. So, it is probable that in these two manuscripts the red color was obtained using these two different chromophores, and that it could only be detected trough molecular fluorescence.

[27] ***Group 2*** - Alc 249, fol. 109v and Alc 419, fol. 98; Alc 427, fol. 115v; Lv15, fols. 16 and 50; Lv50, fols. 1v and 64v, and SC 20, fols. 78 and 86.

[28] *Ms. Bolognese* (15th century), Recipe 130, *To make lake as before in another manner: Take of gum lac 5 lbs., (...) take 6 ounces of verzino in very fine powder* [36].

Regarding the intensities, the variations observed in the various groups are minimal, with lower intensities in group Lac 1, in particular in the excitation spectra with a maxima at 4.2 x 10^5; while in the other groups we find maxima at 4.8 x 10^5, 6.5 x 10^5 and 1 x 10^6, in groups Lac 2, Lac 3 and Lac 4, respectively.

For the historical reconstructions it was found that with a higher amount of alum, the excitation spectrum tends to display more intensity. Be that as it may, although group Lac 1, representing free lac, displays lower intensities than the rest of the groups, the difference may not be sufficient to imply the use or not of alum, with the exception of group Lac 4, which displays a minimum of excitation intensity at 4.6 x 10^5, close to the maximum of excitation of group Lac 1. Generally, the intensity of emission from all the groups are similar. It is the excitation intensity that changes the most between groups. Interestingly, in group SC1, the folios which presented an excitation signal close to group Lac 1, 14v and 24, also present the lowest excitation intensities of this group.

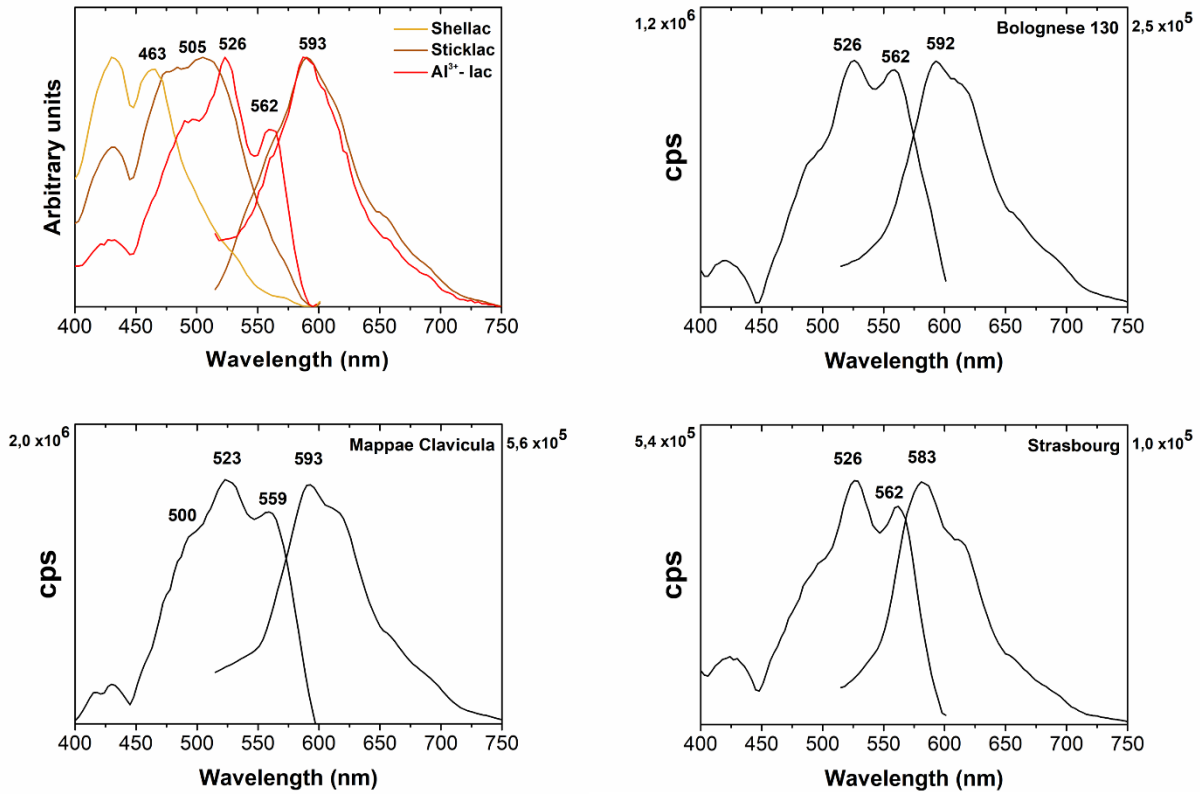

Figure 3.6. Excitation and emission spectra of shellac (processed resin without lac dye chromophores), sticklac (raw material, resin + chromophores), and Al^{3+}-lac complex, pH=3.5; lac dye reconstructions from recipes in *Bolognese* 130, *Mappae Clavicula* and *Strasbourg*, applied using glair as binder on filter paper.

Representative infrared and molecular fluorescence spectra are available in **Table A1.5** - **Table A1.9**. Excepting for Alc 412, fols. 10v and 12 and Lv 13, fol. 30, the infrared spectra of the paints in ***group Lac 1*** show the presence of the shellac resin through its distinctive C-H stretching bands, but the spectra are dominated by the proteinaceous fingerprint of the binder. Fillers such as calcium carbonate and

gypsum are also clearly visible, **Table A1.11**. When compared with the binder, they are usually present in low concentrations, with one exception, the paints in folios 30 and 44v in Lv 13, as it is visible in **Table A1.12**, where the $CaCO_3$ percentage is more than 6 times as higher as the rest of the manuscripts. Most excitation spectra displayed consistently a very broad band with a maximum at *ca.* 472-474 nm and shoulders at 508-514 and at *ca.* 550 nm. On the other hand, the emission spectra display a higher diversity, being characterized by a maximum at ca 590 nm or in the interval 605-615 nm, and a more or less pronounced shoulder at 563 nm. However, no visible correlation between the fillers and the fluorescence emission spectra was detected.

The infrared spectra in ***group Lac 2*** are characterized by a lower amount of shellac resin and a higher proportion of proteinaceous binder and fillers when compared with group Lac 1; Alc 249 and 419 (fols. 109v and 98, respectively) display a high proportion of calcium carbonate and gypsum; Lv 15 and Lv 50 of calcium carbonate and lead white. For the latter, it is possible that the difference in the excitation maxima may be due to the presence of lead white in a relative high amount [11].

In the infrared spectra of the paints in ***group Lac 3*** it is possible to assess the presence of the shellac resin through its distinctive C-H stretching bands, **Table A1.11**. Lv 5, fol.6 display a spectrum dominated by the shellac resin whereas in the other spectra the proteinaceous binder is clearly visible. Gypsum was detected in three paints (Lv 5, fol.6; SC 21 and Alc 247); whereas, in Lv 13, Alc. 421 and Lv 15, calcium carbonate was found, the latter with lead white. Both gypsum and calcium carbonate were employed as fillers (improving the paint mechanical performance) and, depending on the concentration, as opacifiers. So, although the paint formulations differ, the recipe used to produce the lac dye color was similar. In this case microspectrofluorimetry could group the recipes according to the chromophore and infrared spectroscopy was able to discriminate the paints' formulation (SC 21 and Alc 247 are very similar).

In ***SC1*** the infrared spectra are dominated by the protein fingerprint and the shellac resin is not visible, with the exception of folio 14v, similarly to group Lac 1; a small amount of calcium carbonate was detected. There is no information in these spectra that could be used to explain the differences observed in the fluorescence emission spectra.

The two paints in ***group Lac 4*** were found in manuscripts from the collection of the Monastery of Alcobaça, Alc 238 (*Book of birds*) and Alc 347 (*Sermones de verbis Domini*); in Alc 238 paint, the presence of shellac resin and of the proteinaceous binding medium are clearly visible (C-H and amide stretching, respectively), and a high amount of gypsum as filler (when compared with the binder) is also observed. On the other hand, the infrared spectrum of the Alc 347 paint is dominated by the protein fingerprint and a high amount of calcium carbonate. So, again, the paint formulation detected by the infrared spectra did not left a visible mark in the chromophore emission. In this case, as already discussed, the excitation spectrum points to the presence of a brazilwood lake pigment (please, see next section and **Figure 3.6**). Considering that the presence of shellac is very clear in the infrared spectra, we can propose that this paint may be included in the recipes that use a mixture of lac and brazilwood, in the proportion we used to reproduce the *Le Begue* 309 recipe, **Figure 3.6**.

This presence of a brazilwood lake pigment is not visible in the infrared spectrum, although the high gypsum concentration points in its direction [13]. The use of this mixture of lac dye and brazilwood is a very interesting discovery concerning the production and experimentation in the Alcobaça *scriptorium*. It could provide a link from an earlier period in which lac dye paints were used to produce dark reds, pinks and carmine hues to its substitution by brazilwood lake pigments as observed in the winter Breviary Alc 54 dated from the 14th century. A missing link that does not explain why a shiny lac dye paint, was substituted by a very matte color: the same hues are achieved with both chromophores but not the same brightness. This may open new perspectives for an accurate dating of medieval manuscripts.

The chemometric approach for lac dye paints

The results of the cluster analysis using both the excitation and emission spectra are plotted in **Figure 3.7**.This figure offers a general view of the separation of the groups, both for lac dye (**Lac**) and for brazilwood (**BW**). For a close-up of the groups of each colorant please see **Figure 3.8** and **Figure 3.11**. With the exception of the SC1 set, the grouping followed closely our proposal based on the discussion for the fluorescence emission and excitation spectra. Although most of the folia are within the predicted groups, the model had some difficulties in differentiating group Lac 2 and Lac 3, since they share spectral features already discussed, and hence are represented together in **Figure 3.7**.

When observing **Figure 3.7** and **Figure 3.8**, it is noticeable that group Lac 1 is represented at the extremity of the cluster, being considered the most different among the groups, which is in accordance with our preliminary separation, as seen in **Table 3.1**. In the same way, group Lac 4 is the nearest to the brazilwood cluster. This is also in agreement with the hypothesis of a possible mixture of the two chromophores. Because of the use of both excitation and emission spectra, the model correctly predicts the position of this group within the lac dye cluster. Such a prediction would not have been possible using only the excitation spectra, due to similarity with brazilwood paints. Moreover, groups Lac 2 and Lac 3, although being in a separate cluster, are considered the groups more similar to group Lac 4, while SC1, named group Lac 5 in **Figure 3.7**, is a separate cluster, between groups Lac 1 and Lac 2 & 3. This may be due to the excitation spectra of folios 2v, 37 and 77 resembling group Lac 2, with an emission similar to group Lac 1, as discussed in section 1.1.

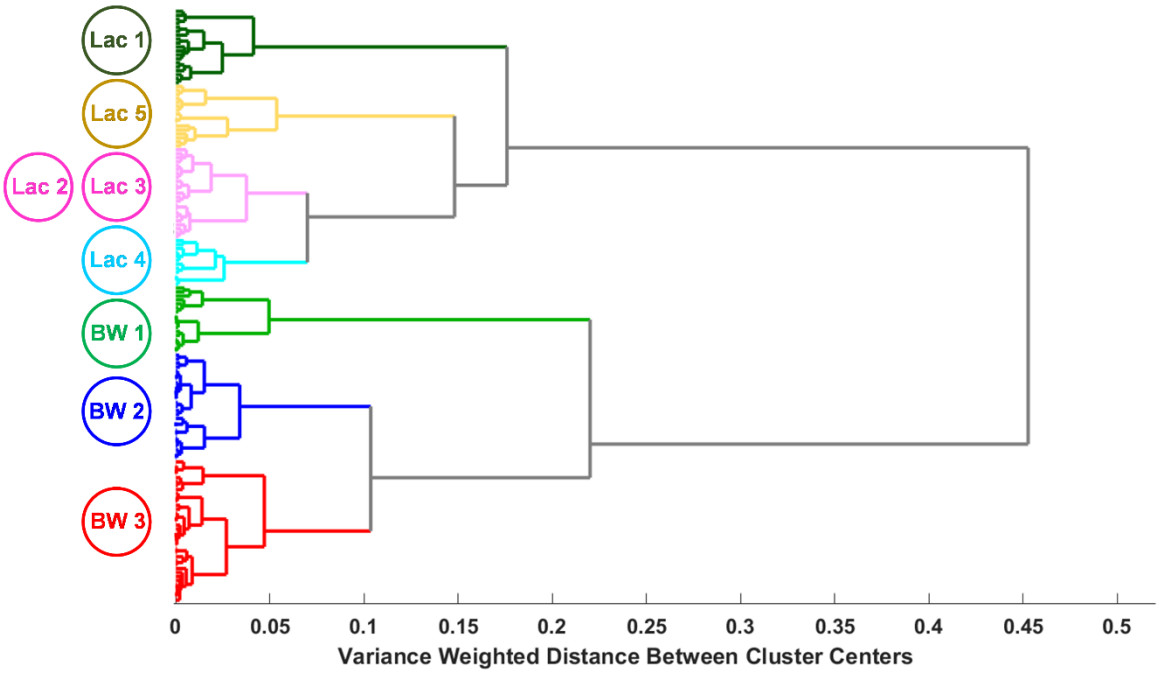

Figure 3.7. Dendrogram generated by HCA applied to excitation and emission spectra of all the historical samples for the lac dye (**Lac**) and brazilwood (**BW**) based paints. With the exception of SC1 (group Lac 5), all the other grouping was predicted by spectral analysis.

Some misplacements can be observed in **Figure 3.8**, when compared with our previous separation of the fluorescence spectra, as described in **Table A1.5** - **Table A1.9**. In group Lac 1, the following spectra are misplaced: SC 21, fol. 2v and Alc 421, fol. 193. The first might be due to a higher intensity of the band at *ca.* 483 nm, when compared to the rest of group Lac 3, where the sample supposedly belong. The placement of Alc 421 in group Lac 1 instead of group Lac 3, may be explained due to poor resolved spectral features, with a higher predominance of the band at 475 nm.

In group SC1, named group Lac 5 in **Figure 3.7**, the following spectra are misplaced: Lv 50, fol. 64v; Lv 15, fol. 26; Lv 13, fol. 21; Lv 12, fol. 94; and Alc 412, fols. 10v and 12. Both the folios misplaced of Lv 15, Lv 50, Lv 12 and Alc. 412 show a shoulder ca 560 nm in the emission spectra, similarly to what is found in SC1, which caused the incorrect prediction within the cluster.

In groups Lac 2 & 3, the only misplaced paint is that of folio 2v of SC 1. It is placed on the cluster Lac 2, indicating some similarity fundamentally between excitation spectra.

Finally, in group Lac 4 the misplaced spectra are: Alc 427, fol. 115v and Lv 15, fol. 50. The predominance of the band at ca. 550 nm, with a well resolved emission spectra with bands at 586 and 614 nm have placed them next to folio 3, lac of Alc. 347.

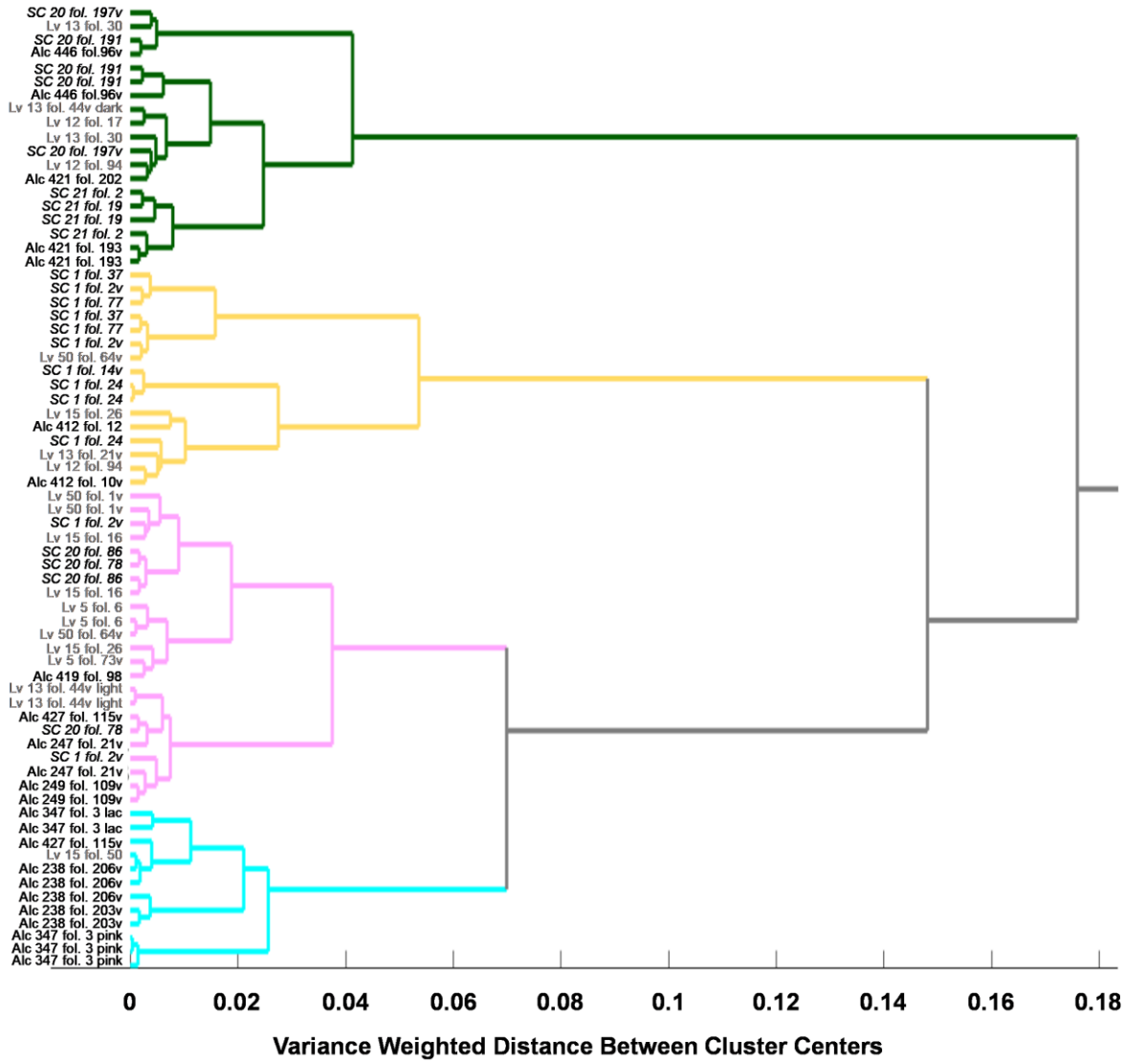

Figure 3.8. Close-up for the lac dye cluster of the dendrogram generated by HCA applied to excitation and emission spectra of all the historical samples of lac dye based paints from all three Portuguese Monasteries: Alcobaça (*black*), Lorvão (*grey*) and Santa Cruz (*italic*): group Lac 1 (*green*), Lac 2 & 3 (*pink*), Lac 4 (*light blue*) and Lac 5 (*yellow*).

3.3.2. Brazilwood in medieval manuscripts

Table 3.2. A total of 80 emission and 80 excitation spectra on 18 brazilwood paints were collected from 8 illuminated manuscripts produced during the 13th c.-15th c. Based on their spectral features these spectra were assembled in 3 groups.

		Group BW 1	Group BW 2	Group BW 3
Excitation maxima		**552-561 nm**	**555-562 nm**	**553-564 nm**
Intensity	Max	2.2×10^5	3.2×10^5	1.7×10^5
	Min	9.1×10^4	1.0×10^5	9.9×10^4
Emission maxima		**582-586 nm**	**594-602 nm**	**590-600 nm**
Intensity	Max	9.5×10^4	8.6×10^4	6.2×10^4
	Min	3.5×10^4	3.0×10^4	2.4×10^4
Infrared		$CaCO_3$ Lead White	$CaCO_3$ $CaSO_4 \cdot 2H_2O$	$CaCO_3$ >> $CaSO_4 \cdot 2H_2O$

Fluorimetric and infrared analysis of brazilwood paints

Contrarily to what was observed with the lac dye paints, for brazilwood pigment lakes, it is the emission spectra that mostly differentiates the paints. They also display a similar intensity, **Table 3.2**. Based on the emission and excitation shapes, we propose to assemble the fluorescence molecular spectra in three groups. In group ***BW 1*** are included all the spectra from Ajuda Songbook, which are characterized by excitation spectra with a maxima within 552-556 nm and an emission maxima at ca. 585 nm, **Figure 3.9**, **Table 3.1** and **Table A1.13** from **Appendix A1.2**. This is in accordance with the reproductions of paints described in the '*The book of all color paints*' as well as the brazilein–Al^{3+} reference sample, see **Figure 3.9** and **Figure 3.10** and **Table A1.17** from **Appendix A1.2**.

The spectra from Books of Hours of French or Flemish production may be assembled in two groups. In group ***BW 2***, the excitation maxima are shifted to slightly higher wavelengths, 555-562 nm, and its shape is less broad when compared with group BW 1, see **Table A1.14**. Emission maxima are also shifted to higher wavelengths, being found in the interval 594-602 nm. The excitation spectra that represent group ***BW 3*** are found between 553-560 nm, falling in the interval of group BW 2, being differentiated by their emission maxima that is shifted to slightly lower wavelengths, 589-600 nm, see **Table A1.15** from **Appendix A1.2**. The differences between these two groups are much smaller than what was found for lac dye paints, and it is possible that they should be considered a single group. For example, IL15 and IL 42 have paints in both groups; this could be due to a different original recipe or to the ageing of the paint. We will further discuss this issue when analysing the infrared data.

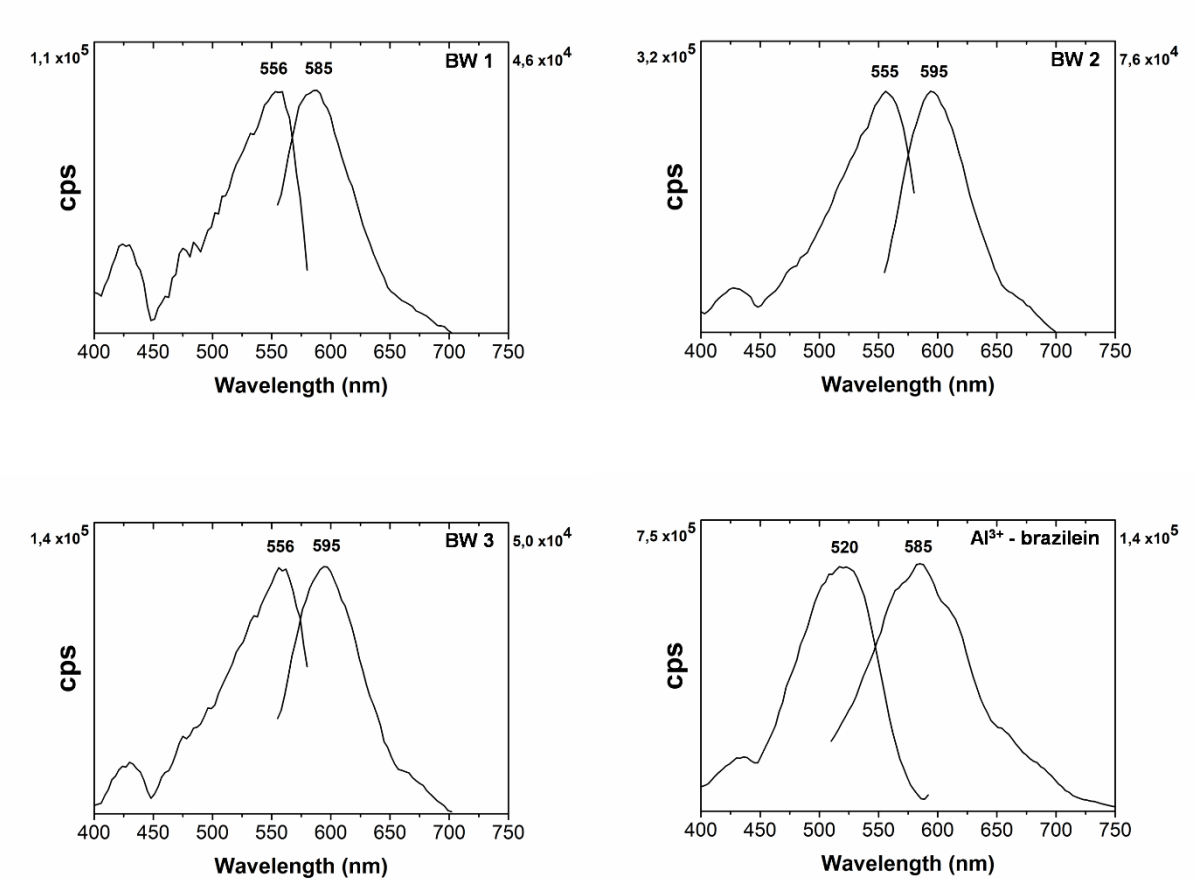

Figure 3.9. Brazilwood paints excitation and emission spectra: group ***BW 1*** (fol. 59 from the Ajuda Songbook), group ***BW 2*** (IL 42 fol. 113), group ***BW 3*** (LH cofre nº 24 fol. 60) and brazilein 5 x 10^{-5} M in MeOH:H_2O (70:30, v/v) with Al^{3+} (x1000) at pH 3.2, applied on filter paper.

Finally, the paints from the Winter breviary (Alc. 54, fol.92) may be compared to the French Book of Hours Cofre nº 22, fol. 76v that is included in group ***BW 3*** (see **Table A1.15** and **Table A1. 16**).

Among historically accurate reconstructions of the four recipes for brazilwood present in '*The book of all color paints*', both excitation and emission spectra display a similar pattern [13]. The excitation maxima are around 550-553 nm and 583-588 nm for emission, see **Figure 3.10** and **Table A1.17**. The emission maxima correlate well with the Al^{3+}-brazilein reference with a maximum at 585 nm, while the excitation is at higher wavelengths than the reference, at 520 nm [13], see **Figure 3.9**.Comparing with the historical samples, the excitation maxima correlates well, although for groups BW 2 and BW 3, a shift of the emission spectra towards longer wavelengths is observed. There is also a good fit of the intensities among the historical samples and the reconstructions.

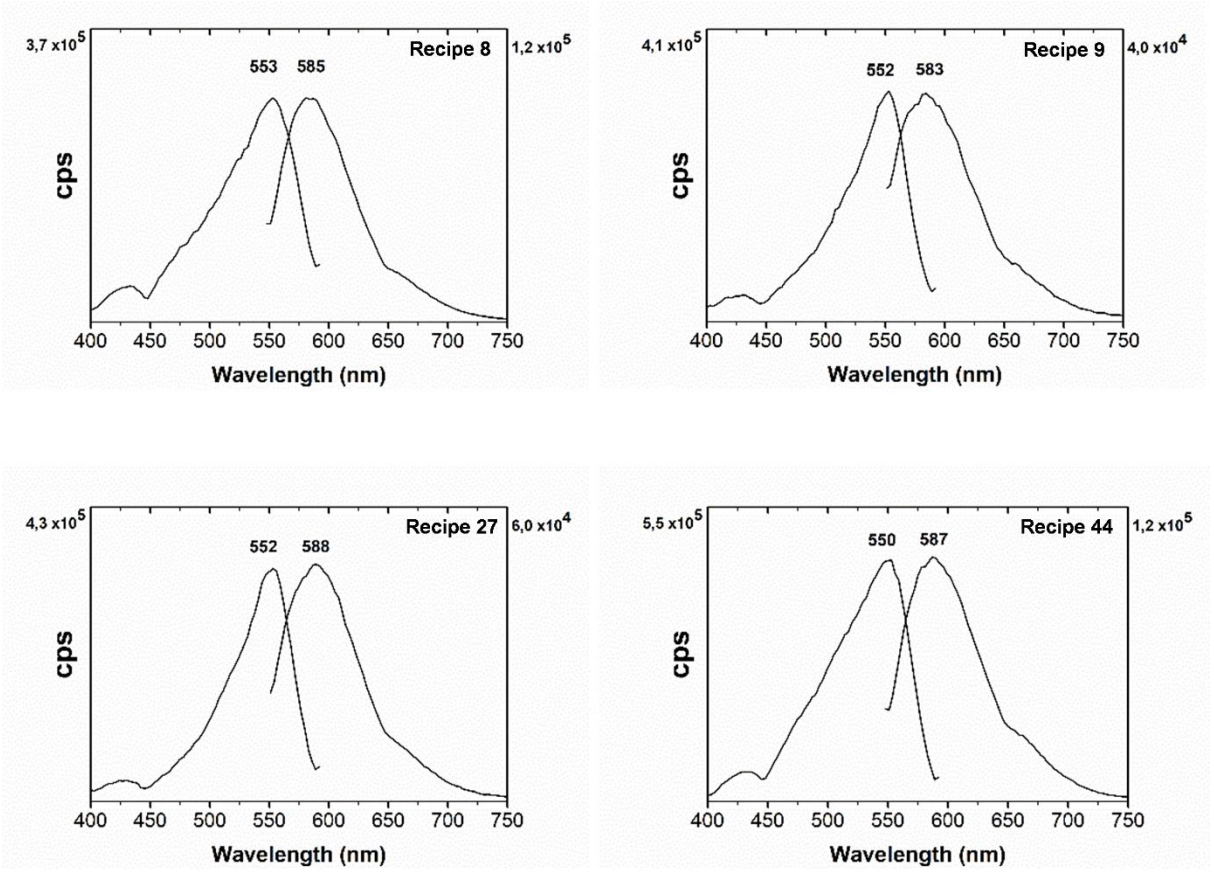

Figure 3.10. Excitation and emission spectra of brazilwood reconstructions from '*The book of all color paints*': recipe 8, 9, 27 and 44.

For both the reconstructions and the original paints, infrared analysis showed the pigments' substrate, extenders and binders. The infrared spectrum of recipe 8 is mainly characterized by the presence of gypsum, while both recipes 9 and 27 present a mixture of gypsum and calcium carbonate. The infrared spectrum of recipe 44 however, is characteristic of the pigments' substrate, showing the aluminate compound [13], see **Table A1.17** from **Appendix A1.2**. The extenders added are known to change the color of the paint. Pigments may appear redder or more violet, darker, or lighter depending on the complexing metal ion, Al^{3+}, Ca^{2+}, or Pb^{2+} [13], with the latter two displaying a bluer shade.

Group BW 1 is characterized by the presence of both calcium carbonate and lead white. This is similar to recipe 8 of '*The book of all color paints*', where the extraction in done with alum and white lead, however, if the filtration is done over a gypsum bowl, no calcium carbonate is present, as seen in **Table A1.17**. This is the only group to which lead white is added, which may explain the low signal-to-noise ratio found in fluorimetry spectra, with the exception of fol. 21 from IL 19, in group ***BW 3***, which also presents a poorer signal.

Both groups ***BW 2*** and ***BW 3*** are characterized by the presence of both calcium carbonate and gypsum. The main difference is the higher amount of gypsum is the latter, which may be responsible for the lower

intensities, see **Figure 3.9**.The infrared spectra of group BW 3 are closely comparable with recipe 9 of '*The book of all color paints*', which is noticeable the presence of both gypsum and calcium carbonate.

Again, the Winter Breviary, Alc. 54, does not fit in any of the proposed groups. The infrared spectrum does not show any added lead white, calcium carbonate or gypsum.

The chemometrics approach for brazilwood paints

The results of the hierarchical cluster analysis, using both the excitation and the emission spectra, are plotted in **Figure 3.11**.The grouping followed closely our proposal based on the discussion for the fluorescence emission and excitation spectra, combined with infrared spectroscopy. The addition of extenders was perceived in the fluorimetry and infrared spectra and by the statistical approach applied. With the exception of IL 19, fol.21, which has lead white, but the emission maximum is shifted towards longer wavelengths in respect to group BW 1. In this case, other additives may be altering the chromophore's environment, causing a shift in the emission spectrum.

Alc. 54, according to the HCA method, was placed within the BW 3 cluster, essentially due to the emission spectrum shifted to longer wavelengths. However, Alc. 54 presents a lower signal-to-noise ratio when compared with the remaining samples of group BW 2. Nevertheless, the clustering method identifies some difference, placing it on the limit of the dendrogram.

Another challenging case is the Book of Hours IL 15, the only one of Flemish production. The three folios analysed are spread through all three groups. The differences observed may be explained because the microsamples were collected on different areas among the several folios.

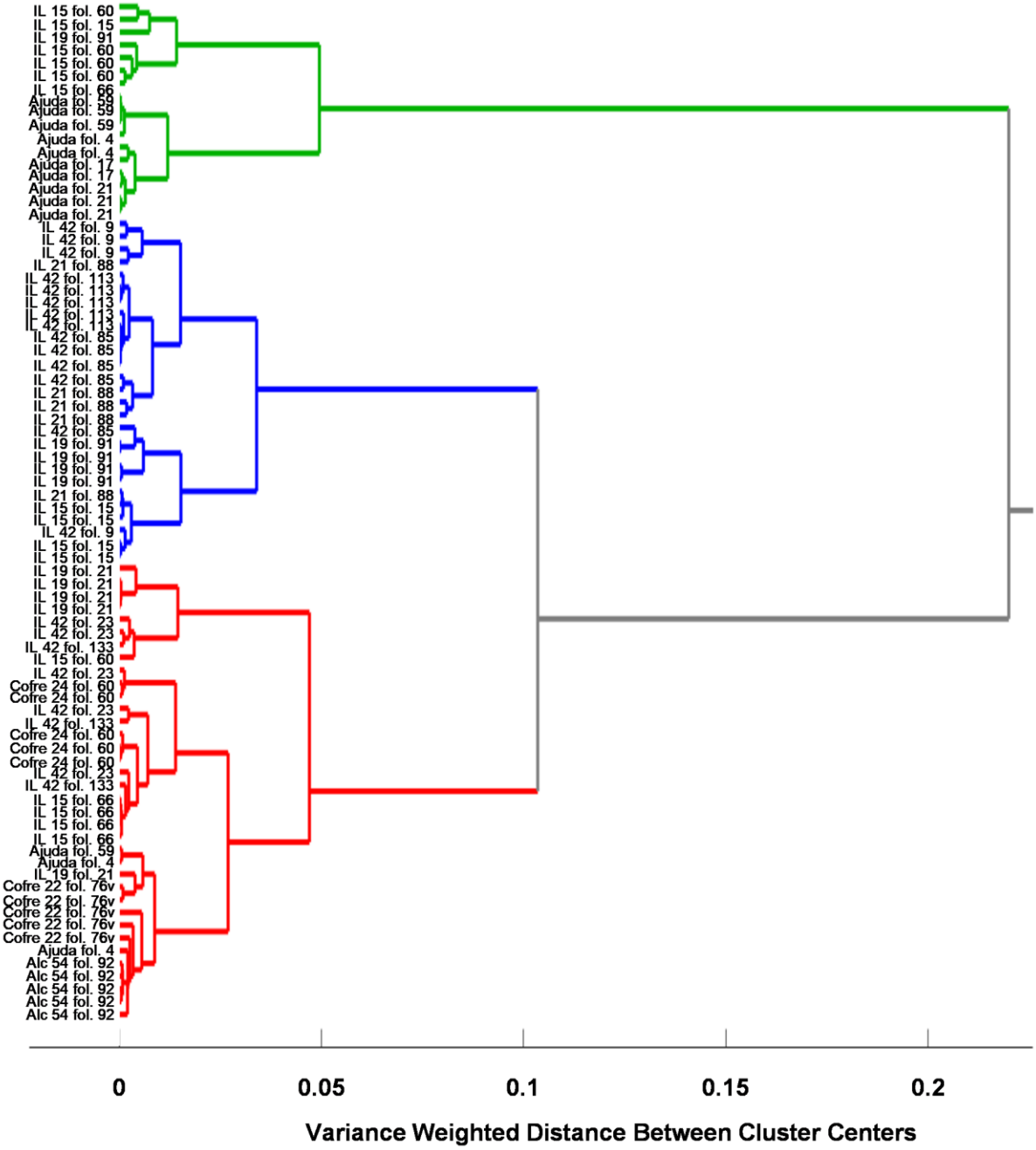

Figure 3.11. Close-up for the brazilwood cluster of the dendrogram generated by HCA applied to excitation and emission spectra of all the historical samples of brazilwood based paints: group ***BW1*** (*green*), ***BW 2*** (*blue*) and ***BW 3*** (*red*).

3.4. Conclusions

Molecular fluorescence spectral data embodies the rich complexity that characterizes paints prepared with organic chromophores. From our knowledge on reconstructing medieval paints we could anticipate that lac dye formulations would be more complex than brazilwood lake pigments. On one side, besides the chromophore, in lac dye paints we may find variable amounts of the shellac resin. On the other, contrarily to brazilwood, from the source material several chromophores are extracted, although we had always found laccaic acid A as a major compound in our paints, **Figure 3.1** [11].

The full discussion of the spectral data in paints previously characterized by a multi-analytical approach, in particular the semi-quantitative analyses of the infrared spectra, was crucial for an in-depth discussion of the fluorescence spectra. The presence of lac dye chromophores had been previously assessed by SERS [11]. Likewise, for brazilwood lake pigments, in selected samples [12, 32].

Overall, the grouping prepared based on spectral data was correctly predicted by chemometrics, with a few misplaced samples, **Figure 3.7**. These misplacements could be easily explained and were either the result of a poor signal to noise ratio or due to its intrinsic difference (when compared with paint reconstructions and the other medieval paints).

Possibly due to the higher heterogeneity in lac dye paints, it was not yet possible to probe the presence of fillers and other additives with the spectral data available. On the other hand, in brazilwood lake paints it was possible to sense the presence of fillers and discriminate between paint formulation. Future work will systematically analyse these effects to provide a general rationale. Importantly, molecular fluorescence pinpointed a formulation in which lac dye and brazilwood chromophores are admixed (group Lac 4), in manuscripts from the Alcobaça *scriptorium* (*De avibus and Sermones de verbis Domini*, Alc 238 and 347, respectively). This could not be observed neither in the infrared nor Raman spectra, in which only the presence of shellac was detected. These paints presented a very good match with a recipe from the text of *Jean Le Begue* (recipe 309). This allow us to hypothesize that, with further developments, molecular fluorescence could be used as a tool to provide geographic information on the place of the manufacture of an illuminated manuscript as well as on its dating.

3.5. References

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Part 2. Characterization of Medieval Manuscripts: From Iberia to Timbuktu

Detail from the Ajuda Songbook, 13th – 14th centuries: Musician, fol. 40v © Ajuda Library

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Chapter 4. Singing with light: an interdisciplinary study on the medieval Ajuda Songbook

Abstract

A monument to Galician-Portuguese medieval lyric, the Ajuda Songbook (Cancioneiro da Ajuda) also contains an exceptional series of illuminations. Employing a multidisciplinary approach, this article uncovers the composition of the color paints used in the Songbook, providing further insight into the circumstances of its production. It also connects the materiality of this manuscript to its economic and cultural context. Our determination of its molecular palette – and the identification of both orpiment and mosaic gold in particular – supports the argument put forward by scholars of medieval literature that the Songbook dates to the end of the thirteenth or beginning of the fourteenth century. We also highlight the extraordinary state of conservation of the lapis lazuli paint, and the presence of a pink color made from brazilwood – the first reported use of this pigment in medieval manuscript illumination. Finally, we discuss the iconography of the Songbook's musical scenes with an emphasis on the depicted instruments. We consider these illuminations and the texts they accompany within their Iberian context (especially in relation to the Cantigas de Santa Maria) and offer a brief comparison with those found in contemporary Occitan and French songbooks.

4.1. Introduction

The Ajuda Songbook (Cancioneiro da Ajuda), which has been kept at the Palace of Ajuda Library, in Lisbon, since 1832, is the only surviving medieval songbook of Galician-Portuguese secular poetry [1]. Despite its renown, many aspects of this manuscript have long remained mysterious. We know nothing of who commissioned it, its date, the location of its production, or the reason why its text, music, and illuminations were never finished. Nor do we know when it was bound with the Lineage Book (Livro de Linhagens do Conde D. Pedro), which opens the codex, or how it ended up in the Royal College in Lisbon (Real Colégio dos Nobres), where it was discovered at the start of the nineteenth century, **Figure 4.1**.We suspect that it spent part of its existence in Évora, an important site of the Portuguese royal courts during the Renaissance, since it was in *Biblioteca Pública de Évora* that, in 1842 a quire and various loose folios from the Songbook were found, and subsequently incorporated into the codex [2]. While valuable as an object in itself, the Ajuda Songbook is also of great importance to the history of Galician-Portuguese lyric, as very few manuscripts compiling these songs survive.

The Ajuda Songbook has been the subject of a wide array of studies since its rediscovery in the nineteenth century, beginning with the pioneering work of Carolina Michaëlis de Vasconcellos, who published a monumental critical edition of the Songbook in 1904 [1].[29] Scholars following Vasconcellos have compared its decorative program with the manuscripts produced at the court of King Alfonso X of Castile and León (r. 1252–84) [2, 3]. But as Patricia Stirnemann has noted, while there are similarities between these works, the Songbook distinguishes itself from its Alfonsine counterparts in a variety of ways [4]. Some experts, including Vasconcellos, have assumed that the Songbook was produced in Portugal, while others have suggested that it may have been produced in Castile in one of Alfonso X's *scriptoria* [5, 6]. The question of its date is also debated: Vasconcellos hypothesized that the project could have started in the final years of the reign of King Afonso III (r. 1248–79), who spent part of his youth in France with his aunt, Queen Blanche of Castile (1188–1252). But given the fact that one of the songs by Paio Gomes Charinho (A 256) could only have been composed after 1287, Resende de Oliveira places its production at a later date [7].[30] Based on the illuminations' style, other scholars have dated the manuscript to the early fourteenth century [8].

Figure 4.1. Lineage Book (pages 1–78) and the Ajuda Songbook (fols. 1–88), including small drawings and inscriptions made in one of the folios that had been left black (fol. 77), are bound together in the Ajuda codex, © Ajuda Library.

[29] The first volume includes the 310 texts of the codex, along with 157 love songs preserved in the sixteenth-century Italian *cancioneiros*, which she believed might have been in the original Songbook. The second volume contains the results of her research on the Songbook and on Galician-Portuguese lyrics, in general.

[30] The song by Charinho is not a love song, but a rather heterodox comparison between the king and the sea, likely related to the author's dismissal from the post of royal admiral in 1287, during the reign of Sancho IV of Castile and León (r. 1284–95).

As many specialists have acknowledged, given the absence of testimonies or coeval sources, questions regarding the manuscript's date and other circumstances of its production might potentially be clarified through the scientific analysis of the materials used to produce its illuminations – that is, the identification of pigments, dyes, binding media, inks, and other details. In addition, various iconographic aspects of the Songbook still require thorough investigation, such as the musical instruments it depicts. This article aims to fill in some of these gaps in scholarly knowledge about the Ajuda Songbook. It conveys the preliminary results of a multidisciplinary study now under way on this codex, in the framework of the research project "*From singing to writing: The Ajuda Songbook, a holistic approach*". This project follows from the earlier international research on Galician-Portuguese lyric funded by the Portuguese Science Foundation (*Fundacão para a Ciência e a Tecnologia*), the results of which have been made available online [9].[31] The present study takes a collaborative and multidisciplinary approach, bringing together the methodologies of art history, technical art history, literary studies, and music history. Each member of our research team brings their expertise to bear on a particular aspect of the Ajuda Songbook, from the molecular characterization of the materials used in its illuminations to its musical iconography. The intention is to gather as much data as possible that might lead us to confirm the date, location, and conditions of the manuscript's production. This research aims to advance our knowledge not only of this particular codex, but of troubadour culture in medieval Iberia more broadly.

4.2. Galician-Portuguese *cantigas* and *cancioneiros*

Galician-Portuguese *cantigas* are among the most distinctive products of medieval Iberian cultural heritage. Composed and sung in the royal and noble Iberian courts over a period of some 150 years – roughly from the end of the twelfth century to the middle of the fourteenth – Galician-Portuguese songs were collected and transcribed in anthologies by the middle of the thirteenth century. The songs are part of a vast cultural movement that Occitan troubadours set into motion at the outset of the twelfth century, and which rapidly spread throughout Christian Europe [10]. Certain characteristics of Galician-Portuguese troubadour culture distinguish it from its Occitan counterpart, such as the development of the *cantiga de amigo* (female-voiced love song), a new genre. Altogether, we have access to some 1680 secular or courtly songs belonging to three genres: *cantigas de amor* (Occitan-style male-voiced love songs), *cantigas de amigo*, and *cantigas de escárnio e maldizer* (satirical songs). These works were written by some 160 troubadours (*trovadores*) and minstrels (*jograis*) who were born in different Iberian kingdoms and territories, but all of whom composed in the language of the peninsular northeast.[32] Galician-Portuguese is also the language of the *Cantigas de Santa Maria*, a set of 420 religious songs praising the Virgin Mary and describing her miracles, which are historically attributed to King Alfonso X.

[31] "Cantigas Medievais Galego-Portuguesas", (also available in English). The database offers the first comprehensive collection of Galician-Portuguese songs, images from manuscripts, music (including original medieval melodies, contemporary versions thereof, and original compositions that take the texts of medieval songs as a starting point), and other resources (Project Littera, PTDC/ELT/69985/2006).

[32] *Trovadores* were noble while *jograis* were non-noble.

Despite their geographic and temporal proximity, the earliest manuscripts of the *Cantigas de Santa Maria* are quite different from those compiling secular songs in Galician-Portuguese, a topic to which we will return.

Secular Galician-Portuguese *cantigas* are primarily known through three manuscripts: the Ajuda Songbook (abbreviated "A"), the *Cancioneiro da Biblioteca Nacional* (National Library Songbook, also known as Colocci-Brancuti, abbreviated "B", cod. 10991), and the *Cancioneiro da Vaticana* (Vatican Songbook, abbreviated "V", Vat. Lat. 4803). These latter two manuscripts, produced in Italy in the sixteenth century, are based upon an earlier songbook, most likely medieval, but today lost. In addition to these three large troubadour collections are two medieval manuscripts that include Galician-Portuguese songs: the late thirteenth-century Vindel Parchment (New York, Pierpont Morgan Library, MS M.979) and the fourteenth-century Sharrer Parchment (Lisbon, Torre do Tombo, ANTT Fragmentos, cx. 20, n° 2) [11].[33] These fragments are especially important because, unlike the great songbooks, they incorporate musical notation. The Vindel Parchment includes seven songs by Martim Codax, six with music, and the Sharrer Parchment preserves seven love songs by King Dinis of Portugal (r. 1279–1325), all with music.

The earliest reference to a Galician-Portuguese songbook is found in the will of Dom Pedro Afonso, Count of Barcelos (c. 1285–1354). Count Pedro was the bastard son of King Dinis and was, like his father, a troubadour. His will, dated 30 March 1350, refers to a *livro de cantigas* (songbook), which Pedro leaves to his nephew, King Alfonso XI of Castile; the book's inclusion in this document and the elite status of its intended recipient indicate that it would have been a valuable codex. What Count Pedro could not have known at the time, however, was that Alfonso had died just days before, during the siege of Gibraltar. It is plausible that Pedro subsequently modified his will prior to his death in 1354 (but a later version does not survive).

The reference to this songbook in Count Pedro's will makes plausible the hypothesis that he owned a songbook commissioned by himself, possibly a songbook based on the alfonsine collection, but including all the later troubadours, if we accept Giuseppe Tavani's argument that a first general compilation had already been made at the court of Alfonso X (King Dinis's grandfather) [5]. In any case, while the will's reference to a songbook is valuable, we cannot with certainty identify it with any of the surviving codices. Both the Ajuda Songbook and the codex that travelled to Rome in the early sixteenth century could in principle be this lost *livro de cantigas*. But, at this point, our lack of data on the Ajuda codex prevents any reliable conclusions.

Be that as it may, the trajectory of the Ajuda Songbook must have been exclusively or mostly Portuguese. The material evidence that this songbook continued to be read in Portugal (or by a Portuguese audience) during subsequent centuries lies in a series of twenty-eight comments written in the book's margins, all of which are in Portuguese, **Figure 4.2**. These inscriptions range from simple words and phrases like fine (*boa*) and excellent (*muito boa*), to playful comments about certain verses.[34]

[33] We do not know if this parchment is a fragment of a medieval manuscript or an isolated bi-folio [11].

[34] As happens in a song (A 18) wherein the troubadour João Soares Somesso says that he cannot rest while a vassal of his mistress lives and the commenter writes *Mata-lo* (Kill him) (when the true meaning of the verse is that this alleged vassal is the troubadour himself).

Palaeographers have concluded that these comments are from four different hands, the oldest dating to the mid-fifteenth century and the most recent to the mid-sixteenth [12]. The Songbook also contains an interesting series of small drawings, inscriptions, and later notations on two folios that had been left blank (fols. 77 and 87), also from the same centuries, **Figure 4.1**.

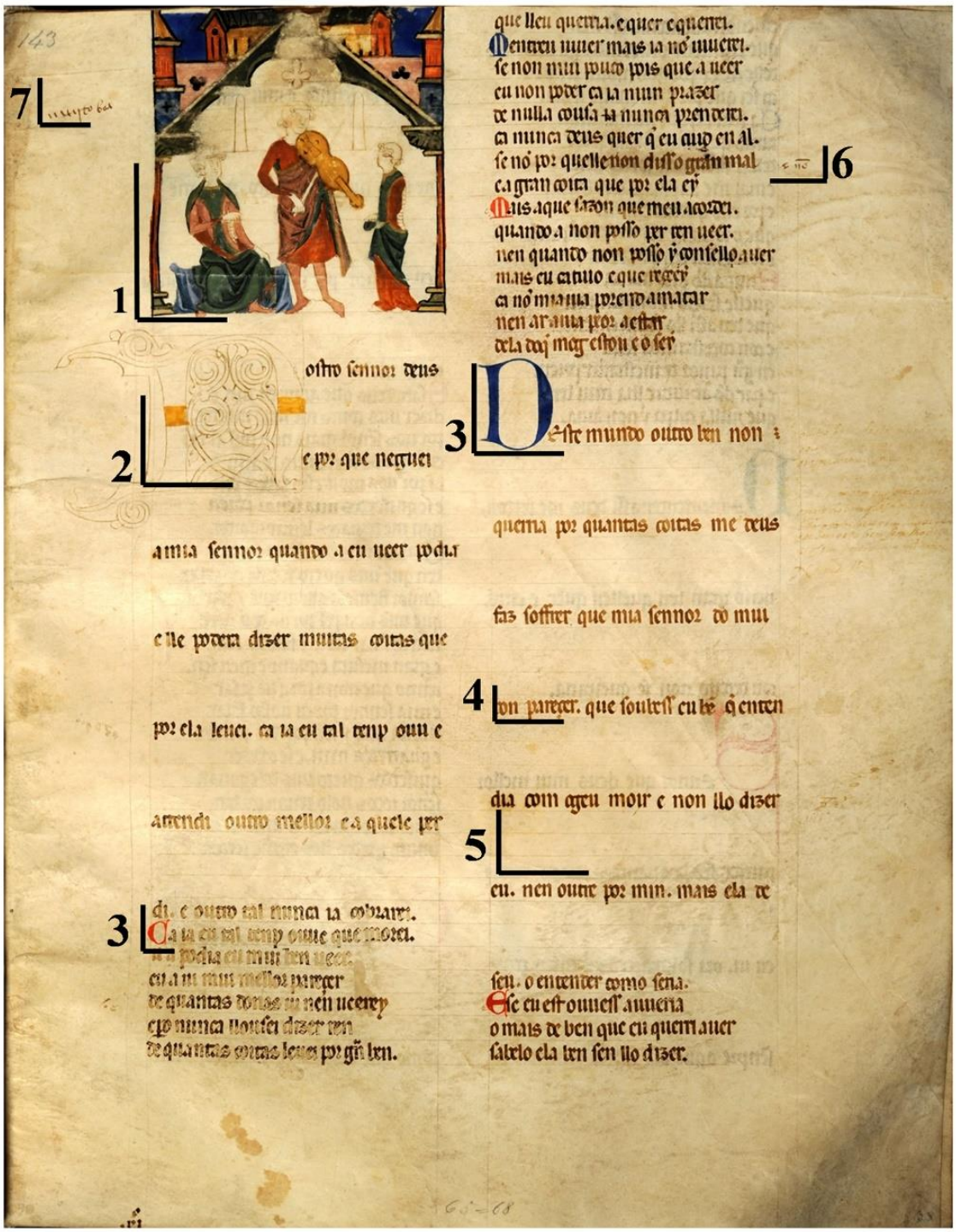

Figure 4.2. Page layout (fol. 33): (1) illumination; (2) decorated initial; (3) larger and smaller initials; (4) song; (5) space for musical notes; (6 revisor's notes; (7) comments made in Portuguese during the 15th – 16th centuries, referring to this song as excellent (*muito boa*), © Ajuda Library.

Contemporary with the last generation of troubadours, the Ajuda codex is a richly illuminated manuscript, yet in terms of the text it is also the most incomplete of the three remaining Galician-Portuguese songbooks. It only contains 310 compositions (of a total of 1680 surviving songs), most of which are *cantigas de amor*.[35] The songs are transcribed in gothic letters that are large enough to be read from a music stand [2]. The names of the authors are not indicated.[36] The manuscript is clearly unfinished; in many illuminations the painting is not complete and in others only the figures were sketched, **Figure 4.2**. The first strophe of each song includes spaces between the lines to accommodate musical notation that was never executed.[37] The reasons why this rich manuscript was left unfinished are unknown to us. But given the information outlined above, we can surmise that the Ajuda Songbook was likely in Portugal through the fifteenth century and at the start of the sixteenth century, and possibly in Évora, as suggested by the discovery of loose folios in that city's library.[38]

4.3. The Ajuda Codex

4.3.1. The Codex

The Lineage Book with which the Ajuda Songbook was bound dates to the late fourteenth century.[39] It is likely that these two manuscripts were bound together in the end of the fifteenth, beginning of the sixteenth century.[40] The Songbook comprises eighty-eight folios, organized in fourteen quires with three extra folios, sewn in when the manuscript was last restored in 2000 [15]. It includes the eleven folios found in the Public Library of Évora (*Biblioteca Pública de Évora*) by Joaquim Heliodoro da Cunha Rivara in 1842 (fols. 4, 16, 17, 29, 36 and 40–5, these last composing quire VII). The folios range from 430 to 442 millimeters in height and 281 to 352 millimeters in width [15]. Some were trimmed at the edges in order to fit the binding. The manuscript presents different numerations, dividing the Songbook in different ways, such as pages or folios, including or excluding the Lineage Book. The present study follows the

[35] We do not know if what was transcribed was intended to be the entire project. The manuscript is clearly incomplete, as several folios are missing; in addition, the last song ends, quite oddly, in the middle of a verse.

[36] Authors' names are documented in B and V, which allows us to identify those in A. Still, the authors of 20 of the songs remain anonymous.

[37] In the remaining strophes this space does not exist, which indicates that the melody was repeated. However, space is also left for the music in some of the *findas* (the short strophes ending certain songs), implying that in these cases the melody would have been different.

[38] Ramos argues that the Songbook might have been in the important library of D. Teodósio (1510–63), Duke of Bragança, located in the palace of Vila Viçosa (not far from Évora): its inventory (1564) documents a volume described as "*Obras del Rey dom Denis feitas de mão de pergaminho de marqua grande em taboa*" [2].

[39] This is a fragment of an expanded copy of the *Livro de Linhagens do conde D. Pedro*, i.e. the aforementioned Dom Pedro Afonso, Count of Barcelos. The original may have been written around 1341–3; José Mattoso dates this copy to 1381–3 [13}. For an edition of this fragment, see [14].

[40] This date is attributed due to the decoration of the binding common to the sixteenth century as well as to a signature of Pedro Homem in folio 88v of the Songbook (previously pasted down in the front wooden board of the binding). This signature is, together with other annotations, from the end of the fifteenth, beginning of the sixteenth century [2, 15]. Conceição Casanova, in discussion with the authors, November 2015.

numeration which divides the Songbook in folios (1–88v), as it separates the latter from the Lineage Book [2].

The bookbinding (end of the fifteenth, beginning of the sixteenth century) is made of animal skin over wooden boards about ten millimeters thick; it is 455 millimeters in height and 345 millimeters in width [2]. The front and back of the binding are decorated with a series of ornamental frames populated with faces in profile and phytomorphic motifs, possibly created by impressing the design onto the cover with a single relief plaque. The two copper clasps (30 x 25 mm) once used to close the manuscript are extant but no longer functional, since only the metal plates mounted in the surface of the wooden boards exist, while lacking the clasps [16]. Two of its folios were formerly pasted down to the inner part of the wooden boards of the binding and were later removed and transferred to the end of the volume (fols. 87 and 88, the latter of which contains songs).[41] In the upper right corner of folio 88v, near the signature of Pedro Homem,[42] is an old shelf number reading "A. 5. n. 47″, written in mid-sixteenth-century chancery script, which could indicate a public or private collection where the Songbook was kept around the end of the fifteenth century. However, it has been impossible to locate such a collection.[43]

4.3.2. The illuminations

Patricia Stirnemann's study of the illuminations of the Ajuda Songbook was recently published in a long-awaited volume stemming from the 2004 conference "*À volta do Cancioneiro da Ajuda*" [4]. For this reason, we will only briefly describe these images, and in another section explore their musical iconography and their relationship to Occitan songbooks.

There are sixteen illuminations, all depicting a musical scene, and twelve blank spaces left for miniatures that were never carried out. All are similar in size (about 10 x 10 cm, the width of the text column), and have a similar composition, **Figure 4.3** and **Figure 4.4**. The contents of the Songbook are organized by author, and each of these illuminations introduces the lyrics of a troubadour. The musicians are placed in architectural spaces characterized by triangular roofs with polylobed edges, supported by colonettes topped with capitals that display a relatively naturalistic acanthus-leaf decoration. Trefoil finials crown each roof, sometimes resembling a fleur-de-lis. Beyond these spaces arise other architectural elements, the exteriors of neighbouring buildings, which provide further spatial context. These microarchitectural frames are composed of towers to each side and in the centre a larger, basilica-type building. As for the figures, Stirnemann notes that - with the exception of the two men found at the beginning of Rui Gomes de Briteiros' compositions - the majority of the illuminations display three characters. These show a man sitting (likely representing the troubadour), accompanied by two musicians or a musician and a dancer (frequently represented as a woman) [4].

[41] This occurred in the last restoration, in 2000 [15].
[42] This Pedro Homem was possibly one of the poets of the Cancioneiro Geral, and a squire in the court of D. João II [2].
[43] Maria Ana Ramos proposes that this can be decoded as cabinet (armário) 5, number 47, or as cabinet A, shelf 5, number 47. She states that it most likely dates between the 1540s and 1560s [2].

Figure 4.3. The sixteenth illuminations all depict a musical scene, as this one in fol. 40v, © Ajuda Library.

Stirnemann concludes that the illuminations are the work of one artist only, evidencing different degrees of completion from image to image: some only display the first layer of each shade, while others are more complete, with highlights and shadows. These tend to become richer and sophisticated, after fol. 29 [4]. Although conveying a different perspective, the latter aspect is also stressed by Ramos:

> "*São justamente as últimas miniaturas que mereceram maior demora na execução do banco em que se encontrava sentado o trovador, diferentemente das primeiras. Este pormenor tem importância, porque desmente a hipótese de o miniaturista ter trabalhado do princípio para o fim do códice, acompanhando o texto e dispondo cada vez de menos tempo para rematar o seu trabalho. Se as suas interrupções significam que o trabalho terminou antes do projectado, então não tratou as miniaturas pela ordem que hoje ocupam no códice*" [2].[44]

[44] "*Unlike the first ones, it is precisely the latest illuminations that required further time in the painting of the troubadours' seats. This detail is important because it refutes the hypothesis that the artist illuminated the manuscript from the beginning to the end, following the text and having increasingly less time to finish his work. If his interruptions mean that the work was finished earlier than expected, then he did not paint the images in the order that they appear in the codex*" [2].

Together with the illuminations, many of the manuscript's ornamental motifs were also left incomplete. The initials that mark the beginnings of the poems, alternately written in blue and red, are decorated with pen-flourished motifs and after folio 14v these initials cease to have the flourishing, possibly due to unfinished work. Also, the decorated initials, which open the first song after the illumination, are never painted and many have not even been designed. Concerning the musical notes, although the text is present, neither staves nor melodic lines were executed, even though space was left for them. The hierarchy in the ornamentation is typical in illuminated manuscripts: the first poem by an author is decorated with a narrative scene and a large initial, while subsequent poems are given pen flourished initials.

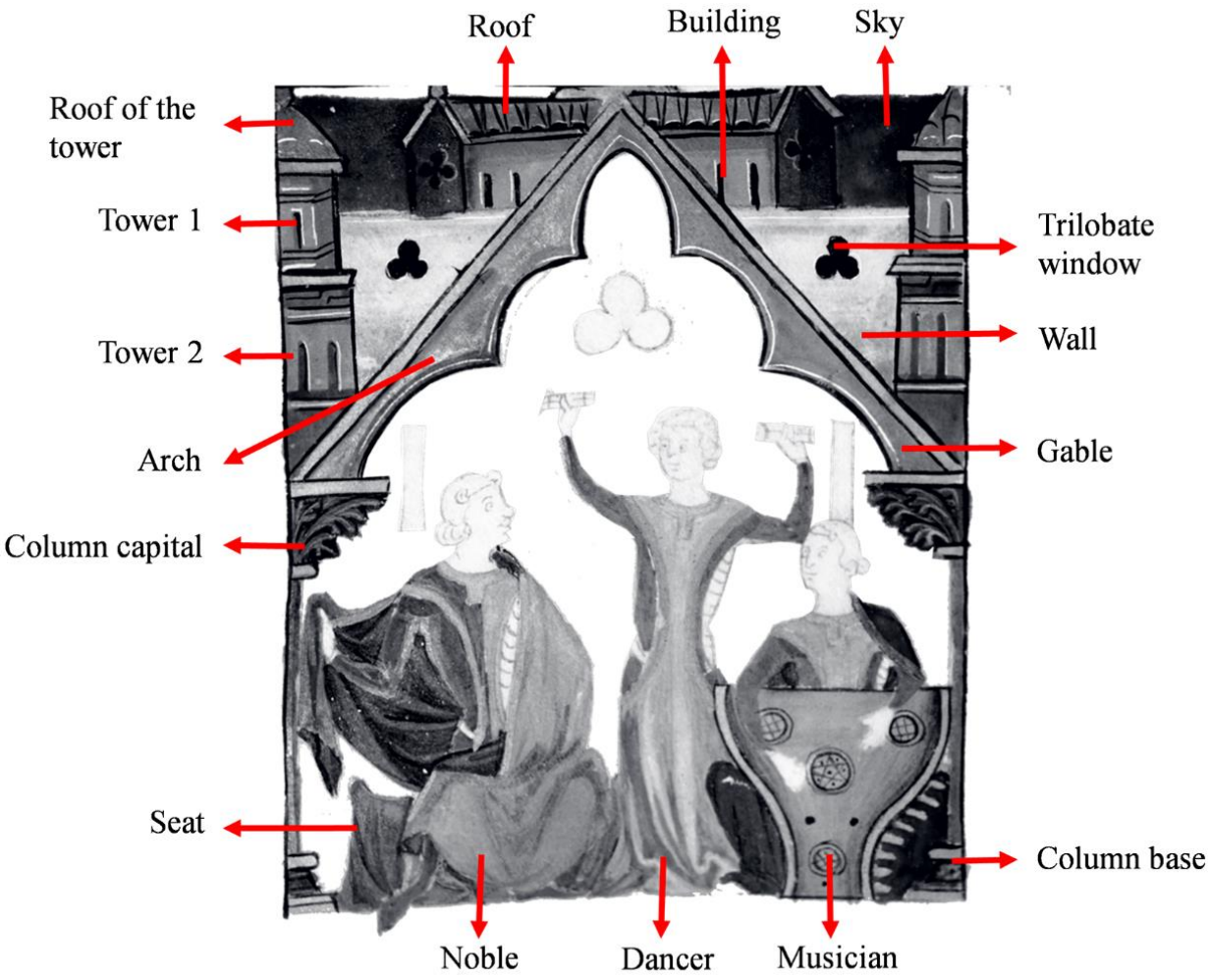

Figure 4.4. Main elements in the sixteenth illuminations, © Ajuda Library.

4.4. Studying the materiality of the Ajuda Songbook: a molecular approach

In recent years we have developed and tested an approach for the molecular identification of the colors used in many different types of medieval codices, from Romanesque Bibles to late medieval books of hours. The goal of this methodology is to determine the precise composition of paints – identifying pigments, dyes, binding media, and other compounds, such as fillers (additives) [17, 18]. This detailed molecular identification is necessary in order to understand the state of conservation of medieval illumination and hence stabilise as much as possible, to prevent deterioration. Over the past ten years, this research was conducted following a three-pronged approach: (i) the creation of new methodologies to identify in situ historical colorants [19-21]; (ii) the study of the formulations of binding media – the "glues" that set the colors over the centuries – and the exploration of statistical methods to address their complex signals [22, 23]; (iii) the creation of a database of references for colorants, binders, and color paints [19, 23, 24]. Here, we will briefly describe how the molecular characterization of colors was carried out.

Paints are first analyzed by optical microscopy to understand how the final color was built up, to detect possible degradation phenomena, and to select which colors will be subjected to a more detailed characterization in the laboratory in order to identify the colorants, binders, and additives. After examining the illuminations with the naked eye and under the microscope, we begin the molecular analysis of the color paints and inks. Given the complexity and value of these materials the analytical techniques are generally interfaced with a microscope. This enables us to study areas on a micro scale – a micrometre (µm) being one thousand times smaller than a millimetre. But the molecules we need to characterize are much smaller and can only be "seen" at the nano-level – a nanometre being one thousand times smaller than a micrometre. These molecules can nevertheless be detected through spectroscopic signals, known as spectra. Spectroscopic techniques deliver a spectrum, which can be understood as a simulacrum of the real molecular world; this complex image is interpreted by comparison with reference databases and by decoding the signal obtained as much as possible.

Our analysis combined elemental with spectroscopic techniques. By detecting the elements present in the pigments, micro X-ray fluorescence (microXRF) allows us to identify the colorants and, in certain cases, it enables the semi-quantification of pigment mixtures through comparison with reference materials.

Raman microscopy (microRaman), Fourier transform infrared spectroscopy (microFTIR), emission fluorescence spectroscopy (microspectrofluorimetry), and fibre optic reflectance spectroscopy (FORS) in the UV-visible region are powerful complementary spectroscopic techniques for the characterization of colorants in medieval manuscripts. Raman and infrared spectroscopy reveal a "*molecular fingerprint*": if a single compound is present, it is possible to unequivocally characterize it using these techniques. Complexity can arise, however, because we are usually faced with aged, superimposed mixtures of

compounds. Another layer of difficulty is added when a compound is not present in our reference database.

During the in-situ investigation of the Songbook, the first screening was carried out by FORS and microXRF, the latter of which indicates the colorants and extenders present and allows for the initial quantification of these elements; moreover, the 70 µm offered by microXRF enables us to obtain data on the distribution of a certain paint color throughout the manuscript. Because microRaman and microspectrofluorimetry allow for high spatial resolution (1–5 µm spot), diverse paint components were analyzed separately. Combining SERS (surface-enhanced Raman spectroscopy) and microspectrofluorimetry was paramount for a full characterization of the dyes present. FORS analysis was carried out for all sixteen of the main illuminations, enabling us to extend the results obtained from a detailed molecular analysis to six selected folios (fols. 4, 16, 17, 21, 40v and 59).

The criteria used for the selection of the areas of analysis have been described elsewhere and the folios studied may be consulted in **Appendix 3**. In the laboratory, samples were also analyzed by microFTIR, allowing us to characterize binders and gain insight into the full paint formulation.

4.5. Color in the making

4.5.1. The materials and techniques of medieval illumination

The materials used to produce a color paint include the colorant, binding medium, and additives. Given the lack of technical studies on the colors used in the Ajuda Songbook or coeval manuscripts that might be comparable to it, we here discuss how these materials may contribute to dating a medieval illumination. By accurately characterizing color paints in context,[45] it is possible to propose chronologies for the use of colorants in medieval European manuscripts, and systematic studies have been successfully carried out along these lines. Examples include Claude Coupry's work on the *scriptorium* of Fécamp Abbey in the eleventh and twelfth centuries, and our own investigation of colors in Portuguese monastic manuscripts from the twelfth and thirteenth centuries [17, 18, 25-27]. Coupry's molecular characterization of the blue pigments used in the *scriptorium* of Fécamp allowed her to propose a general chronology for the use of the color blue [28]. In a geographically broader context, based on the collection of the Fitzwilliam Museum, Paola Ricciardi and co-workers proposed a chronology for the use of greens in medieval manuscripts [29].[46]

Our own investigations of color materials in Portuguese monastic manuscripts and French books of hours in Portuguese collections have led us to argue that organic colorants are one of the most important

[45] Within a specific region, at a specific *scriptorium*, or during a specific period.

[46] Considering that the greens were identified by visible reflectance spectroscopy (FORS), a technique that does not provide unmistakable molecular characterization, caution must be taken with respect to this proposed chronology.

materials for establishing a chronology, in particular the red hues: carmine,[47] pink, and purple [18, 30-32]. [48] Therefore, based upon studies that have characterized the red dyes used in medieval European manuscripts, and also upon a critical analysis of medieval technical treatises, we find the following chronology for the use of red/carmine/purple colors [30, 31, 33-36]: lac dye red was used in monastic *scriptoria* during the twelfth and thirteenth centuries, while brazilwood-based carmine and pink colors were used from the fourteenth century on, in both monastic *scriptoria* and lay workshops [31, 36-39].[49]

With respect to inorganic pigments, the chronology of the use of yellow is perhaps the most revealing. The synthetic pigment known as mosaic gold may have been introduced in the thirteenth century [40].[50] Variations in its production can provide important clues as to when and where a color paint was prepared [41, 42]. Based on the results of studies of European manuscripts, we can draw the following conclusions about the use of inorganic yellows: orpiment was used in monastic *scriptoria* from the eleventh to thirteenth centuries [18]; mosaic gold was used possibly from the end of the thirteenth century or beginning of the fourteenth century; and lead tin yellow began to be used in the fifteenth century [31].

Although we cannot offer a precise date for the introduction of brazilwood pinks and mosaic gold yellows in manuscript illumination, we can ascertain, through unequivocal material analysis, that they were being used in the fourteenth century [43, 44]. This is also confirmed by technical treatises like the *Liber diversarum arcium*, the *Book on how to make colors* (*Livro de como se fazem as cores*), and *De arte illuminandi* [45-47]. It is possible that these colors were already available for use in manuscripts at the end of the thirteenth century, but we have found no information in the literature to prove this.

4.5.2. The materials of color in the Ajuda Songbook

Colorants

In **Figure 4.5** we present the molecular palette used in the Ajuda Songbook.[51] Reds were painted with vermilion, minium, and brazilwood pigment lakes.[52] Yellows were produced with orpiment, mosaic gold,

[47] Carmine is used thorough the text as “hue”, the attribute of color perception denoted by carmine, and is not related with the molecule carminic acid.

[48] Purple hues that extend past a more or less dark red also having a blue color component: which is to say our colorant typically absorbs between 500 and 550/600 nm.

[49] Before the colonization of Brazil by the Portuguese, which began in 1500, redwood was imported to Europe from Ceylon (Sri Lanka). Its presence in European trade peaked in the fifteenth to sixteenth centuries. In medieval technical treatises this color was referred to as “brazil” or a similar term.

[50] In the treatise De arte illuminandi, dated by Franco Brunello to the end of the fourteenth century, mosaic gold is indifferently designated as *aurum musicum*, *purpurina*, and *purpureus color*. The author of the treatise proposes that the designation “mosaic gold” derives from the fact that it was used “*a dorare le tessere dei mosaici*”, but there is no explanation for the term *purpureus color*. The pigment obtained following the recipes in medieval treatises, such as the *Livro de como se fazem as cores*, gives a brilliant metallic, golden-copper color, provided that it is not over-milled; it is therefore interesting that in Portugal and Spain, metallic pigments are still commonly referred to as *purpurinas*. See *purpurina* in [40].

[51] In **Appendix 2** are shown the spectral data used for the full paint characterization.

[52] Minium was applied in the vestments of the nobles. As in the Portuguese Lorvão Beatus, many of the Songbook illuminations present degradation of this pigment, where a transformation from bright orange to brownish hues is visible. For more details on red lead degradation in medieval illuminations see [48]. Vermilion was applied in the roofs of the architecture, in the vestments, and in several initials.

and yellow ochre, with the orpiment primarily used to create greens. For the blue colors we find lapis lazuli and indigo.[53] Blues are always provided by the precious lapis lazuli, appearing on architectural details and on clothing [49]. It is shaded using dark blue indigo and carbon black. Carbon black was also used for outlines and small details.

Figure 4.5. The molecular color palette used in the Ajuda Songbook.

Except for the greens, the paints are applied as pure colors (that is, not admixed with other colorants), highlighted or lightened using lead white. Carbon black and indigo, in combination with orpiment yellow, are used to create green, the only color that is not based on a single colorant but is instead produced by combining a dark color with yellow, **Figure 4.6**. Combinations of a yellow with a blue or black, also known as *vergaut,* are described in detail in the *Liber diversarum arcium* under yellow-green ("*If you want to make yellow-green. Mix orpiment with black, matiz with orpiment.*") and green ("*Put orpiment and indigo or azure, makes yellow-green, incide with indigo tempered with vinegar or with azure such that it should be more green, matiz with orpiment*.") [45].[54] These methods of making green are likewise used in the Ajuda Songbook. A combination of orpiment yellow and blue is used for architecture as well as clothing. A combination of orpiment yellow and black is used exclusively on the vegetal elements that decorate the capitals, in a delicate *matiz*, where the artist shades and highlights by meticulously applying orpiment over the black underlayer,**Figure 4.6**.

[53] We detected copper on the blue initials, which may indicate the presence of azurite as an underlayer; however, it was not possible to acquire any further molecular evidence for its use.
[54] Recipes [§1.27.11A] and [§1.27.35].

Figure 4.6. Combining orpiment yellow with indigo blue or carbon black to obtain "*green*/*yellow-green*," which are applied in the vestments (top) and architecture (bottom).

The diversity of colors, accentuated by the presence of lapis lazuli blue and brazilwood pink, demonstrates the luxuriousness of the color palette [50]. Lapis lazuli was detected by Raman microscopy, which identified its color center. Its characterization was complemented by infrared spectroscopy, which shows that the color center is found in an aluminum silicate matrix, and that it was applied in a proteinaceous tempera, **Figure 4.7**.

The pink color produced using brazilwood pigment lakes was the most challenging to characterize, as is typical for a dye-based color. Evidence of its use is found by cross-referencing the information in the excitation and emission fluorescence spectra with the reference database of brazilwood pigment lakes we have built by consulting medieval treatises [31, 33, 34]; the detailed characterization of the main chromophore, brazilein, is discussed in **Appendix 2** and in [51]. This pink is one of the main colors found in the Songbook, where it is normally applied as a light pink in architecture as well as clothing. It is also found shaded with a protein varnish that saturates its color, **Figure 4.8**. Additives were used to further alter its appearance: gypsum was added to render it opaque, while a lighter hue was created by admixing lead white. The darker pink is found in the clothing worn by the noblemen, as on folio 59. Although there are records that brazilwood was imported from Southeast Asia into Europe in the twelfth century [52] – so much so that we are aware that it was used to dye textiles and was a fashionable color among the

French aristocracy[55] – there is no evidence for its application in European manuscripts before the fourteenth century.

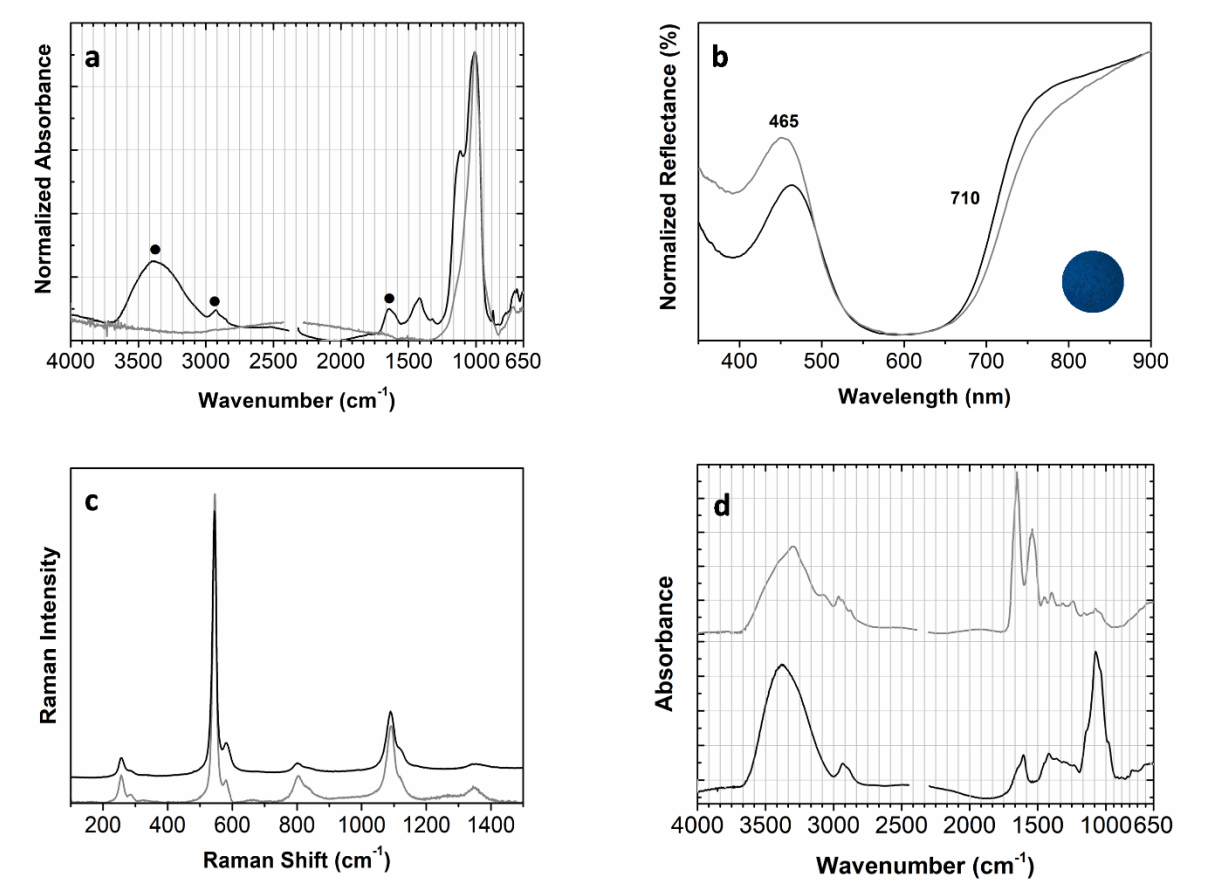

Figure 4.7. Lapis lazuli is used in the skies (background), and architectural elements and vestments, as proved by its molecular spectra. Spectra for lapis lazuli reference (*grey*) and blue in a tower, fol. 4 (*black*): (*a*) infrared; characteristic peaks for protein (●), possibly in mixture with a polysaccharide; (*b*) FORS; (*c*) Raman, λ=632nm; (*d*) infrared reference spectra of protein (*grey*) and polysaccharide (*black*).

With respect to the colors' state of conservation, if on the one hand the lapis lazuli blue used for the backgrounds is in an exceptionally good state, on the other we observed darkening in the lead-based pigments, **Figure 4.9**. While we have frequently detected the degradation of lead white and minium in our studies of Portuguese medieval illumination, the good state of conservation of the lapis lazuli is highly exceptional [17, 48, 53].[56] We often observe the loss of lapis lazuli paints by detachment, which may be associated with a low binder/pigment ratio [38]. In future projects, we intend to investigate why the blue of the Ajuda Songbook is so well-preserved. Its pristine surface and high chromatic saturation could be due to a different formulation of the binding agent, a better-quality stone that allowed a finer grain, thereby leading to a better binder/pigment ratio, or a particular preparation of the support.

[55] It is not considered a lightfast textile dye, so its success must have been due to either a special taste for this color or its rarity, given the small quantities imported in the thirteenth century.

[56] It would be worth pursuing a comparative analysis with the lapis lazuli colors applied in English medieval wall painting [53]. We thank Patricia Stirnemann for pointing out these examples of application of lapis lazuli in mural paintings to us.

Figure 4.8. The pink color is based on a brazilwood pigment lake and was profusely applied in the vestments (*top*) and architecture (*bottom*).

Binding media

In addition to establishing the chronology of the use of certain colors, we also aim to determine the chronology for the use of binders, the invisible components of the paint. Our studies show that in Portugal, protein temperas were used in the twelfth and thirteenth centuries, while polysaccharide temperas or mixtures were used in the fifteenth century [39].[57] Corroborating our findings with regard to fifteenth-century books of hours, the author of De arte illuminandi (writing at the end of the fourteenth century) supplies us with an excellent summary of the binding media used in the practice of illumination:

> "*Dei liquidi con cui si temperano i colori per fissarli sulla carta: I liquidi con cui si fissano i colori sono questi: cioè l'albume e il tuorlo d'uovo di gallina, la gomma arabica e la gomma*

[57] Examples of proteins include egg white (glair) or yolk, parchment glue, and casein glue; polysaccharides include gum arabic, mesquite gum, and gum tragacanth.

adragante disciolte in acqua limpida di sorgente. Talora a raddolcirli, è necessária l'acqua di miele, o di zucchero o di candito…" [47][58]

Most of the Ajuda Songbook's colors were applied using a polysaccharide as a binder, but at this stage it is not possible to accurately identify it, **Figure 4.10**. It is important to mention the use of glair as a varnish to saturate the light pinks and yellows, producing color and light effects that have survived to this day, **Figure 4.11**: medieval illuminations with their original varnish are quite rare. This demonstrates a mastery of color and an advanced technical knowledge of how particular materials alter the properties of color paints. Given the artist's high level of expertise with materials, we propose that the binder used for the lapis lazuli blue may explain the unusual state of preservation of this color. Attempts at reproducing this paint are under way using casein glue recipes.

Figure 4.9. Degradation in the Ajuda Songbook (details). Lead white darkening (*top*) and the increase in surface heterogeneity that accompanies the darkening in red lead paints (*bottom*).

[58] "*On the solutions used to temper the colors and fix them to the parchment: the solutions [liquids] used to fix colors are as follows: chicken egg whites and yolks, gum arabic and gum tragacanth dissolved in clean spring water. In order to make it less brittle, honey, sugar or crystalized sugar water is added…*"

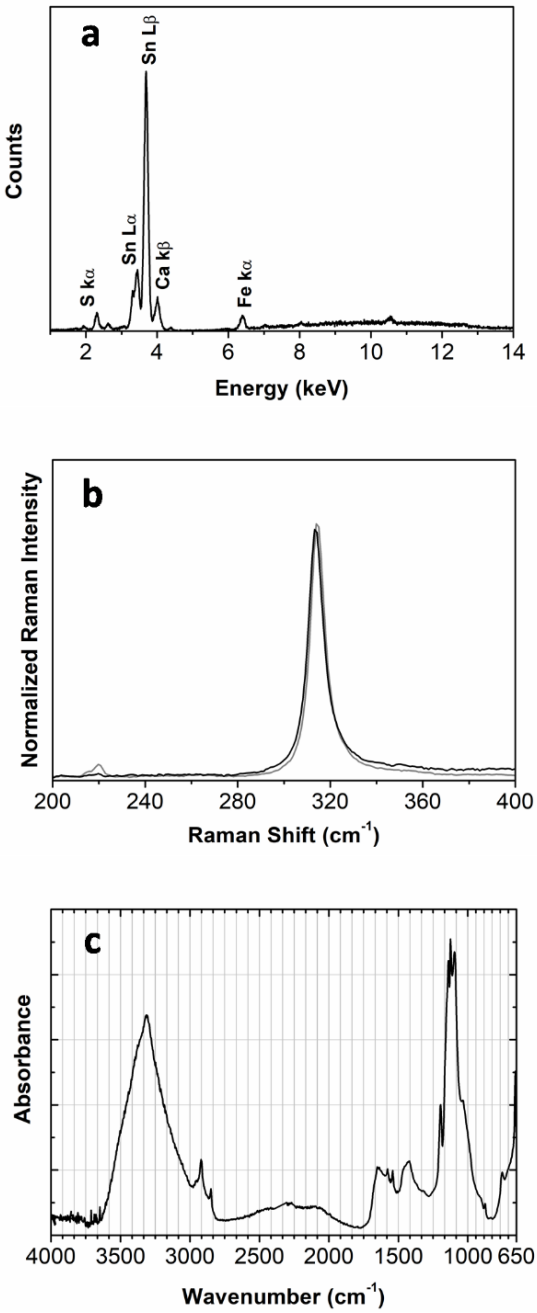

Figure 4.10. Mosaic gold was used in architecture and vestments details, as proved by its molecular spectra. Representative data for mosaic gold reference (*grey*) and the golden color found in architectural elements in fol. 4 (*black*): (*a*) μ-EDXRF; (*b*) Raman spectra, λ=630nm; (*c*) infrared spectrum, possibly of the binding medium (yet to be identified).

Figure 4.11. Examples for the use of the yellow in architectural details: general image showing the use of both pigments (l*eft*) which are mosaic gold (*middle image*) and ochre (*right*).

Writing ink

A semi-quantitative analysis of the writing ink, using microXRF data, was undertaken to assess the different hands at work as assigned by Ramos [2]. Elemental analysis shows that in all inks the main element is iron, indicating the use of iron-gall ink.[59] Although it has not been possible to distinguish between the writing inks used by the different hands identified by Ramos, we found that the ink used on quire VII (the folios found in Évora) is different due to the presence of zinc, which accompanies the main element, iron, **Figure 4.12**[2]. Differences in elemental composition were also observed in some of the reviser's ink, in which copper is present; in this case, we analyzed only three folios, and further analyses are planned using a larger sample set. In the drawing of the illuminations and illuminated initials, however, only iron was detected in the ink; in this case, we did not observe a different elemental composition in the Évora quire. This elemental composition was again found in the inks used on the small drawings and in the Portuguese comments that appear in the margins, except on folio 77, where we also found an iron-copper ink.

The materials of color and the dating of the Ajuda Songbook

The scientific analysis of the pigments and other materials used in the illuminations of the Ajuda Songbook proved helpful in our efforts to date and geographically locate the production of the manuscript. The way yellow and green colors were applied – specifically, the use of orpiment and mosaic gold – is particularly revealing in this regard. Knowing that poisonous orpiment, as a yellow hue, was discontinued in the fourteenth century and that it could have been replaced by yellow ochre also present in the Songbook palette, its use points out that it was specifically chosen for the greens. Also notable is

[59] Iron gall ink can be obtained by adding an iron salt to a tannin solution, or by adding a mixture of an iron and copper salt. These elements may be accompanied by lower amounts of other metallic elements (such as zinc) that allow us to distinguish between the compositions of various types of writing ink, even when the color is indistinguishable to the naked eye. In the case of the Ajuda Songbook, the distinction is made based upon the proportions of iron (main element), copper, and zinc.

the use of ochre to create “pure” yellow colors, which is not common in the medieval European illumination we have studied; in books of hours the iron-based pigments are generally applied as a brownish “wood” color for furniture, floors, and walls. However, in the fourteenth-century winter Breviary from the Alcobaça monastery (also studied within the scope of this special volume), the only yellow aside from gold leaf is an ochre with a similar molecular fingerprint. Could its use be regarded as specifically Portuguese or Iberian? Only a larger sample size will allow us to answer this question, and we anticipate that this will be an exciting path to pursue.

It is worth noting that the *Liber diversarum arcium* – which Mark Clarke suggests was largely written around 1300 – had text added throughout the fourteenth century, up to the point when it was transcribed into the surviving manuscript from around 1400 that we know today (c. 1400–30) [45]. It contains three entries for yellow: orpiment (*auripigmentum*), saffron yellow (*crocus*), and ochre.[60] Orpiment and ochre are used in the Songbook, but saffron is absent.[61] Mosaic gold, which replaces saffron in this triad, is not mentioned in this treatise, despite the fact that it is one of the most complete testaments of artistic practices in the age of transition from illumination to the oil painting of the great Flemish masters.[62] This absence is perplexing and may be explained by the fact that the central portion of the treatise was produced around 1300, when mosaic gold was not yet widely available as a pigment [42]. If future studies confirm the unavailability of mosaic gold before 1300, this would date the production of the Ajuda Songbook as later than 1300. On the other hand, if the manuscript could be precisely dated to the end of the thirteenth century, based on documentary sources, it would be the earliest occurrence of this pigment in European medieval books.

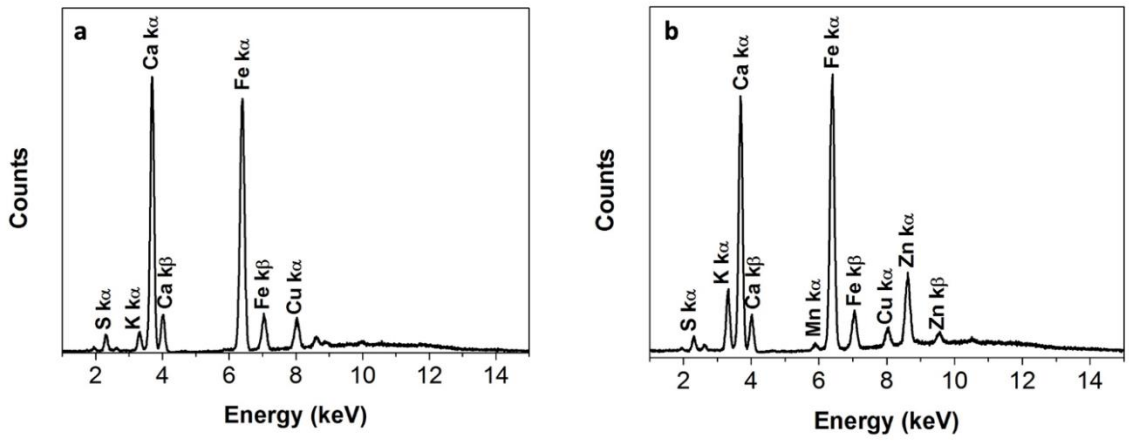

Figure 4.12. Representative µ-EDXRF data for writing inks: (*a*) fol. 4; (*b*) fol. 40v. The presence of zinc in fol. 40v indicates the use of at least two different inks.

[60] “*There is found another yellow color which is called ‘ochre’: namely that found in the region of Chiavenna and in many places, that is to say it is a sort of earth, but that which is brought from the town of Tours is the most valuable then others*…”, [45], recipe [§1.20.1].
[61] It is possible that saffron could have been applied as a glaze, but it can pass undetected if it is in a degraded state. See [54].
[62] “*It is probably the most substantial and comprehensive mediaeval painters’ technical recipe book to survive and summarizes the state of the art in the European workshops of the fourteenth century*” [45].

If the presence of brazilwood pigment lake indicates a fourteenth-century date for the Ajuda Songbook, the use of orpiment yellow could push the date back into the thirteenth century.[63] In the Songbook, mosaic gold serves to emulate powdered gold (Figures 10 and 11) [55]. Although its production in the Middle Ages is described in technical treatises from the fourteenth and fifteenth centuries [47, 56], its identification in medieval manuscripts is quite rare.[64] Although it has been found in a thirteenth-century polychrome sculpture,[65] only in fifteenth-century manuscripts has mosaic gold been identified [31, 44, 55, 57, 58]. In the books of hours we have studied, it was often used as a substitute for pure gold [57].[66]

4.6. Iconographic analysis of the Ajuda Songbook

One of the biggest challenges in studying the Ajuda Songbook's illuminations has been establishing comparative analyses with coeval works. No other secular illuminated codex from Portugal like it has survived.[67] However, this absence does not preclude the possibility that the Songbook was produced in Portugal, because we have found no other works stylistically and ichnographically comparable to it from outside Portugal either. From the time of its discovery in the nineteenth century, many attempts have been made to find commonalities between its iconography and that of other manuscripts, particularly those produced at the court of King Alfonso X el Sabio. We propose that comparisons with French and Occitan troubadours' songbooks from the period must also be considered. These are the questions we will discuss in this section.

The musical instruments

Some musical instruments depicted in the manuscripts compiling the *Cantigas de Santa Maria*, specifically the so-called *Códice de los Músicos* (Biblioteca de El Escorial MS B. I. 2) and the *Códice Rico* (Biblioteca del Monasterio de el Escorial T-I-1), provide an indisputable commonality between the Alfonsine codices and the Ajuda Songbook. A careful analysis of the instruments depicted in the illuminations of the Ajuda manuscript is therefore essential.

The instruments that appear in the Songbook's musical scenes are mostly chordophones (stringed instruments): the harp is present in three illuminations; the viola also in three illuminations; two types of

[63] Possibly owing to its deadly toxicity, orpiment was gradually replaced by other synthetic yellows such as mosaic gold and, later, by lead tin yellow.

[64] "*Through analysis, these samples were identified as the extremely rare and overlooked pigment mosaic gold [….] This discovery is particularly interesting because mosaic gold has rarely been identified in actual artworks, even though it is mentioned in numerous original recipes of the fifteenth century*" [55].

[65] Thirteenth-century polychrome stone statue of Santa Ana in Santa Maria la Real, Sasamon, Spain [57].

[66] It is worth mentioning that in fifteenth century books of hours, lead tin yellow admixed with blue was used to make green, and to highlight or shade the green color. In the Ajuda Songbook this was done using orpiment. See [31, 59].

[67] We assume that the codex to which the Sharrer Parchment belonged was not illuminated. The great earthquake that destroyed Lisbon in 1755 (including the royal palace and its library) is the most commonly used justification for the loss of any Portuguese historical document or manuscript.

psaltery, in four illuminations; and the citole (an ancestor of the guitar) in eight illuminations, **Figure 4.13**.[68] Percussion instruments also appear: round tambourines are present in three illuminations and flat castanets in four illuminations. Contrary to Alfonsine manuscripts (Ms. B.I.2 and Ms. T-I-1), however, there are no wind instruments [60-62].[69]

The harps represented are all similar to one another, and exhibit the characteristics of Romanesque harps, with a decorated column, console, and curved sounding board. According to Rosario Álvarez, the harp was already known and represented in French iconographic sources in the twelfth century, but only rose to prominence in Iberia during the fourteenth and fifteenth centuries [60]. Harps are frequently depicted in thirteenth-century illuminations, especially in Bibles and Psalters, where they are frequently associated with King David as the author of the Psalms; however, these manuscripts typically show the gothic harp, and it is always drawn on a smaller scale. The model of harp found in the Ajuda manuscript is not very common in thirteenth-century illumination, but two instruments of this kind are found in the aforementioned manuscript of the *Cantigas de Santa Maria*, the *Códice de los Músicos* (Ms. B.I.2, cantiga 380, fol. 341). Furthermore, the Maciejowski Bible (also known as the Morgan Bible)[70] – illuminated in or near Paris in the mid-thirteenth century – shows a harpist (fol. 26v) whose instrument is very similar in shape to those in the Ajuda Songbook (fols. 15, 37, 47); of note is the cloverleaf- shaped opening in the sounding board, which also appears in the French manuscript. Additionally, the flat castanets that appear in the Ajuda Songbook (fols. 4, 16, 17, 29, 51v, 55, 59) are shown in women's hands on three folios of the same French Bible (fols. 29, 39, 39v), although this is not the only source that depicts this idiophone.

The violas shown in the Ajuda Songbook are not oval, as is common in France and in the Alfonso X manuscripts. They are instead shaped like a figure eight, with an accentuated curvature. The size of the instrument is clearly too large, given its scale relative to the player. The illuminator appears to have had difficulty transferring the models, likely from a sculpture, to the reduced scale of the illumination, likely drawing it from memory.

Certain iconographic elements might be interpreted as indicative of the influence of Alfonsine manuscripts, such as the citole in the illumination of the prologue to the *Códice Rico* (Ms. T-I-1, fol.5) and Ms. B.I.2, fol. 29.[71] However, the recurring presence of minstrels playing violas in the Songbook leads us to dismiss this proposition, since this type of bowed string instrument is not found in any of Alfonso's manuscripts.

The citole is most commonly found in Iberian iconography; for instance, they are held by two of the Twenty-four Elders of the Apocalypse in the relief sculpture at the Collegiate Church of Santa María la Mayor in Toro (Zamora, Spain). A musician playing a citole can also be seen on a corbel of the Gelmírez

[68] See Rosario Álvarez's discussion of similar instruments in the codices of the Cantigas de Santa Maria [60]. About the cítola/citolón, see [61]. To know more about the identification of musical instruments see also [62].
[69] It is interesting to note that in a satirical song by Martim Soares (B 1357, V 965), which is addressed to a bad troubadour and musician, the use of trumpets (and also drums) is mentioned as evidence of his poor and uncourtly artistic qualities.
[70] New York, Morgan Library, Ms. M.638.
[71] Minstrels with citoles, on the right, the same type of instrument found in the Ajuda manuscript, in the illuminations on folios 18, 29, 40v, 47, 48, 51v, 55, 60.

Palace in Santiago de Compostela. Two more appear among the sculptures at the Monastery of San Lorenzo de Carboeiro (Galicia); despite their poor state of conservation, one can identify the characteristic configuration of the resonator (soundbox) and openings, small holes in the upper lid, the sound holes, above and below, two on each side. Within the Portuguese context, in addition to the Ajuda Songbook illuminations, we only find this chordophone (though its identity is uncertain given the image's poor state of conservation) in the depiction of musical angels in the large window of the Collegiate Church of Guimarães, but this dates to the early fifteenth century.

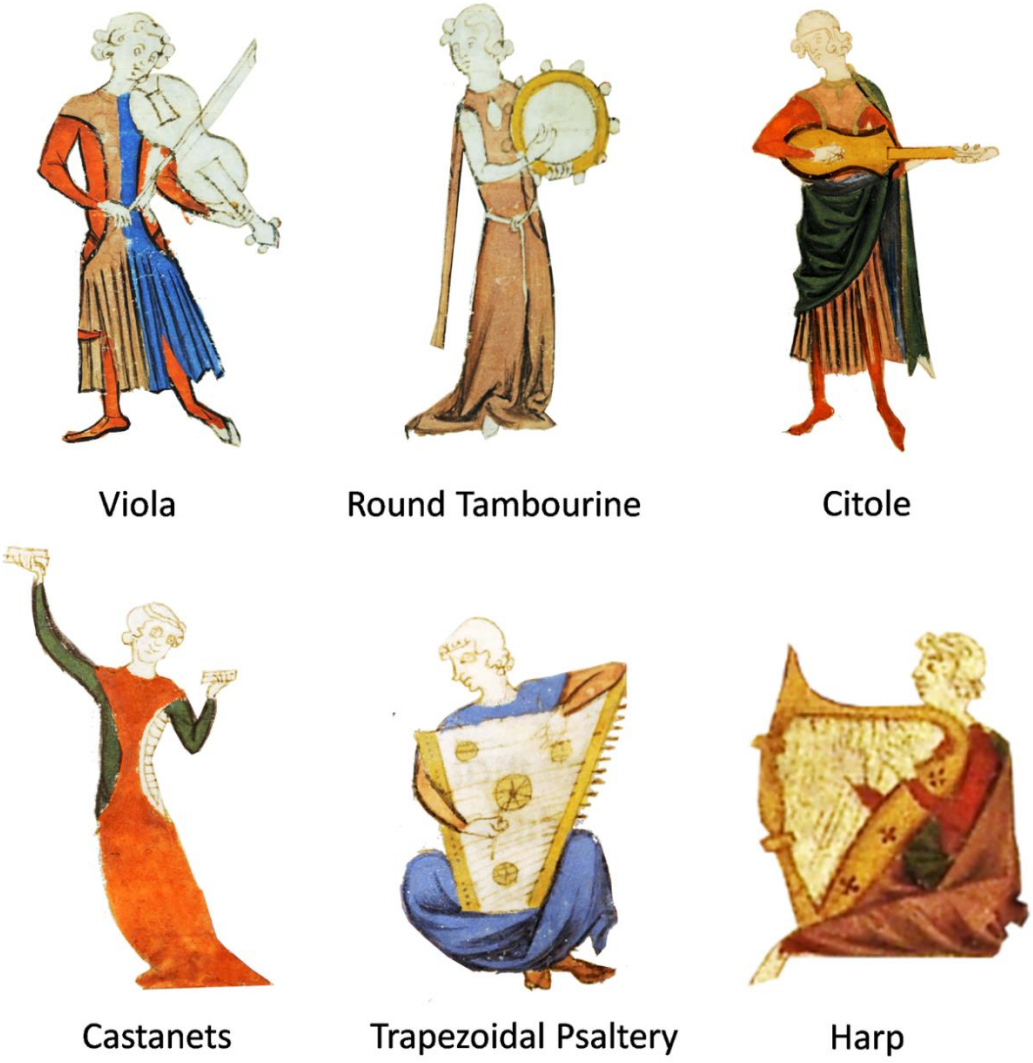

Figure 4.13. Musical instruments depicted in the Ajuda Songbook, © Ajuda Library.

Since the spaces provided for the musical notation were left blank, it would be incautious to infer relationships between the instruments represented and the intended or actual execution of the songs. The fact that almost all of the texts in the Ajuda Songbook are *cantigas de amor*, a genre usually in the *gravitas* tone (i.e. more serious and majestic), makes it unlikely that they were intended for dancing, though that activity is present in many of the illuminations. Thus, the scenes convey a certain cultural

environment where the poetic-musical genres were cultivated and should not be regarded as an accurate depiction of a "real" performance. Moreover, we also observe certain organological errors in the design of the instruments, such as inaccurate dimensions or, in the case of the illumination on folio 37, the positioning of the harp strings between the neck and the column, whereas they should be between the neck and the soundboard.

The figures

In addition to the musical instruments, the figures depicted in the Ajuda Songbook's illuminations also deserve a closer look. In her analysis of the garments worn by these figures, Patricia Stirnemann determined that they are characteristically Iberian [4]. She notes in particular the unpainted laced slit (*fente lacée*) in the left side of the surcoat, as in the illumination on folio 17, a detail also present in the *Cantigas de Santa Maria* (Ms. B.I.2 and Ms. T-I-1). She observes that this detail disappears in the fourteenth century. Stirnemann also points out that two citole players and two viola players (fols. 21, 37, 40v, and 55v) wear surcoats that are cut into strips at the bottom, revealing their legs. She proposes that these strips of fabric would likely have produced a subtle rustling sound [4]. Although absent from Alfonso's codices, this type of garment was fashionable across Europe during the last decades of the thirteenth century and appears in several manuscripts including two mentioned above: the Maciejowski Bible (fols. 17 and 29) and the Barcelona Haggadah (fol. 61) [4]. But although these iconographic similarities between the Songbook, Bible, and Haggadah are quite remarkable, these three manuscripts are quite different in terms of their respective styles of painting.

Although the *Cantigas de Santa Maria* and the Ajuda Songbook share a similar Iberian context, and even the same language, each had a distinct purpose and function (sacred *versus* secular). On the other hand, despite their linguistic difference, in terms of textual content the Ajuda Songbook is closer to the secular Occitan and French songbooks (*chansonniers*) produced from the mid-thirteenth to the fourteenth century.[72] It is important to stress that the Ajuda Songbook is roughly contemporaneous with the emergence of these collections of the works of Occitan troubadours and French *trouvères.*[73]

Each of the Ajuda Songbook's illuminations appears at the start of a series of texts by a particular author, whom it depicts as a seated figure [2]. The intention was not to create portraits that convey the individualized facial and physical features of these poets, but rather to evoke their presence and underscore their importance as the authors of the corresponding works. Contemporary Occitan and French songbooks offer iconographic parallels in this regard. An Occitan songbook from the second half of the thirteenth century, likely produced in northern Italy (Chansonnier I; Paris, BnF, Fr. 854), depicts

[72] The earliest dated Occitan songbook is manuscript D (Modena, Biblioteca Estense, alfa.r.4.4]), from 1254. The structure and organization of the Ajuda Songbook also follow that of its European counterparts in that the works are organized by author/troubadour in a roughly chronological way. See Paden, [63]. He adds: "*Manuscripts D and V [1268] are the only two chansonniers that bear specific dates prior to the fourteenth century*."

[73] Troubadours wrote in the southern French (Occitan) dialects, including Provençal and Limousin. *Trouvères* wrote in the northern French dialects – that is, the *langues d'oïl* as opposed to *langue d'oc*.

troubadours in the illuminated initials of the corresponding texts; they are generally represented as isolated figures dressed as nobles or armoured knights on horseback). References to musical performance are generally absent.

Another Occitan songbook produced in Italy (Chansonnier M; Paris, BnF, Fr. 12474), which dates to the fourteenth century, apparently depicts a troubadour seated within an architectural space defined by two trefoil arches; he extends an index finger towards an open book placed upon a lectern, a gesture that suggests he is the author of the words it bears (fol. 1). Contrary to the figures in Chansonnier I, his placement and gestures recall the seated figures in the Ajuda manuscript, a similarity that supports their identification as authors.[74] The depiction of musicians accompanying troubadours is uncommon among Occitan and French songbooks. One example can be found, however, in the fourteenth-century Occitan songbook known as the Songbook La Vallière (Chansonnier R; Paris, BnF, Fr. 22543).[75] In its first historiated initial (fol. 1), we can identifya seated musician, certainly the troubadour, playing a trapezoidal psaltery, a chordophone similar to that on folio 16 of the Ajuda Songbook. In the bottom margin of the same folio in the Songbook La Vallière, one finds two minstrels, one playing a lute and the other an oval-shaped viola, the most common type at that time.

Authors' "portraits" are sometimes present in *trouvère* manuscripts as well.[76] As Ramos points out, the figures' authorship is indicated by their postures, gestures, and even clothing [2]. These images of troubadours and *trouvères* sometimes include "biographical" details or signifiers of identity, as in the religious attire given to clerics and the women's garments given to *trobairitz*. Others reflect the content of a certain song, as in another image of Thibaut de Champagne, preceding his song "*Feuille nem flor nem vaut riens en chantant*", where he is shown pointing to a tree covered with leaves (Paris, BnF, ms. Fr 846, fol. 53v).

References to authorship are not so clear in the Ajuda Songbook, where the seated figures seem to depict the authors in a less specific or "biographical" way. Although unfinished the Ajuda Songbook, due to its dimensions, *mise-en-page*, and decorative and iconographic program is a rich codex; in this sense it is perhaps comparable only to the early fourteenth-century German Manesse Codex (Heidelberg, University Library, Cod. Pal. germ. 848), which is decorated with a series of 137 illuminations depicting authors, including many complex narrative scenes.

[74] Folio 89 shows a hybrid straight flute and drum hybrid in a rather fantastical composition; fol. 165 contains what appears to be a gallant scene, without musical elements; also on the footer of fol. 235v there is a fantastical hybrid figure playing a viola.
[75] Although the compositions are rather different from those in the Ajuda Songbook, both manuscripts are organized by author; R contains a significant number of melodies (161) written in tetragrams outlined in red.
[76] Represented mostly in historiated initials, as in the late thirteenth-century Songbook Cangé (Paris, BnF, Fr. 846), where, among others, Thibaut de Champagne is depicted writing down a song (fol. 94r). This manuscript is organized, however, by the songs' alphabetical order.

4.7. Conclusions

The conceptual models of the Ajuda Songbook are Occitan and French songbooks, but the illuminations' iconographic and formal models have an Iberian matrix, as evidenced by the similarities we found with other examples of Iberian illumination as well as sculpture. We do not have coeval Portuguese images that are stylistically or thematically similar to the Songbook's illuminations, nor do we have sufficient information to propose the existence of a *scriptorium* connected to the Portuguese court. However, there were certainly illuminators in Portugal who would have been perfectly capable of making a manuscript of this quality.

The manuscript's date remains uncertain. The molecular characterization of its colorants points to a date around the beginning of the fourteenth century, particularly the use of mosaic gold and brazilwood paints. However, other data imply that it might date to the end of the thirteenth century. This evidence includes: *(i)* the fact that the latest troubadour included in the compilation is Paio Gomes Charinho, who died in 1295; *(ii)* the Iberian sartorial detail of the *fente lacée*, which was fashionable in the thirteenth century but fell into disuse in the fourteenth; *(iii)* the presence of orpiment, which was discontinued in the fourteenth century.

Importantly, the chromatic palette used in the Ajuda Songbook demonstrates the desire and the resources to produce a sumptuous manuscript. Although the illuminations exhibit varying degrees of completion, the architectural background is clearly finished and the faces may have been purposely left unpainted so that the color of the parchment would come through. It is possible that the backgrounds were also meant to be left as bare parchment, as in the *Cantigas de Santa Maria* (*Códice Rico*, Ms T-I-1).

It thus seems clear that the Ajuda Songbook was produced within an Iberian context, and read by a Portuguese audience, given the presence of marginal notes in the Portuguese language. A hypothesis proposed by specialists is that it may have been sponsored by King Dinis or his son Pedro Afonso, Count of Barcelos. Pedro's intellectual profile and his interest in courtly literature (he was even a troubadour himself) would have provided the justification and the resources for a project such as this. Even if the Ajuda Songbook is not the book of songs mentioned in his will, his ownership of (and therefore interest in) a book of this type suggests that he might have commissioned one at some point. The poetic-musical compositions of troubadours and *trouvères* began to be collected and transcribed in songbooks during this very period, and the involvement of the Portuguese court in Occitan and French culture – especially during the reigns of King Afonso III and King Dinis – make it plausible that a luxurious songbook might have been produced in Portugal for an elite or royal audience. Nevertheless, it is still possible that a Galician patron may been responsible for the Ajuda Songbook.

We now plan to pursue new lines of research in order to achieve a better understanding of the color chronology of brazilwood and mosaic gold paints in medieval manuscripts. Yellow ochre may also provide important clues, since it will eventually be possible to ascertain with some precision from where it was excavated. These future studies must also include additional comparative analyses of

contemporary Iberian illuminated manuscripts and European songbooks, as well as the further analysis of pigments in Alfonsine codices, particularly the *Cantigas de Santa Maria* (*Códice de los Músicos* and *Códice Rico*).

4.8. References

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Chapter 5. Organic reds in Islamic manuscripts (12th – 15th c.) produced in Al-Andalus

Abstract

The conservation of Islamic manuscripts in the Fondo Ka'ti created the opportunity to study the organic red colorants applied in five manuscripts, which include a Koran (1198), a Theology Treatise (14th c.), a Book of Poems from Al-Sarishi (15th c.), a Biography of the Prophet (1468) and Manuscript 19 (1485). These dark red colors were characterized using fibre optic reflectance spectroscopy (FORS-VIS), microspectrofluorimetry and infrared spectroscopy (microFTIR). Microspectrofluorimetry detected the presence of a lac dye chromophore in all the manuscripts studied and ascribed it to specific medieval recipes for three of the manuscripts. This was based on the very good matches obtained with our database of paint reconstructions that were prepared according to medieval technical sources; the dark reds found in the Koran compared very well with the recipe 'to make red ruby from lukk' from Ibn Bādīs text (11th c.); the brighter reds applied in the Book of Poems and in the Biography of the Prophet, with recipe 113 from the Paduan manuscript (16th c.). MicroFTIR completed the characterization of the paint formulation, identifying the proteinaceous nature of the binding media as well as the fillers. It also showed the presence of oxalate compounds, possibly, resulting from the binding media degradation, a mark of the recent and dramatic history of these books. Finally, these red dyes were successfully compared to lac dye colors previously characterized in 12th-13th c. Portuguese manuscript illuminations. From Mali to Iberia, tracing the rich diversity of a precious heritage legated by medieval Arabic culture.

5.1 Introduction

The rescue and conservation of a group of Islamic manuscripts, from Timbuktu, Mali, created the opportunity to study the materials and techniques used to illuminate these medieval books [1-4]. In this work, we focused on the molecular characterization and degradation assessment of the red dyes. The organic purples applied in the Theology Treatise are described in **Appendix 2** and all the inorganic pigments will be discussed in another publication although a general table is provided in **Appendix 4**. These manuscripts are now preserved at the Timbuktu Andalusian Library (*Biblioteca Andalusi de Tombuctu, Fondo Ka'ti*), and its president, Ismael Diadié Haïdara, dated them between the 12th c. and the 15th c. and made a first classification as follows; a Koran (1198), a Theology Treatise (14th c.), a

Book of Poems from Al-Sarishi (15th c.), a Biography of the Prophet (1468) and Manuscript 19[77] (1485). Thereafter referred to as Koran, Mss 19, Al-Sarishi Poems, Prophet's Biography and Theology Treatise. Except for the Koran, applied on parchment and paper over parchment, all other manuscripts are written and illuminated on paper support. This research will increase our knowledge on the materials and techniques of manuscripts produced in al-Andalus, particularly in the use of historical dyes.

5.1.1. The manuscripts of *Fondo Ka'ti*

The five manuscripts were produced either in Andalusian territory or under the Arab diaspora. Until 2012, the manuscripts were stored in Timbuktu's libraries [5-9], when their integrity was at risk due to the imposition of the Sharia law by a faction of the Tuareg tribe. It was possible to rescue more than 370 000 manuscripts, in a plan coordinated by Abdel Kader Haïdara, and for which many people risked their lives for the safeguard of their cultural heritage [6-8]. To avoid their destruction by the faction of the Tuareg tribe, these works were hidden and transported in dramatic conditions [9].

5.1.2. State of art on the characterization of organic reds in Islamic Manuscripts

Dye characterization in medieval illuminations is a challenging endeavour [2-4,10, 11], as we need to address the fundamental chromophore as well as the full "formulation" to get in-depth knowledge about the culture that produced these precious colors - which are centuries or even millennia - old [12, 13]. High performance liquid chromatography with diode array detection and mass spectrometry (HPLC-DAD-MS) combines high separation power with a complete dye characterization, but only in exceptional cases it will be possible to have a micro-sample from an illumination with enough material to be extracted for HPLC. For this reason, for dye identification, our group devised other approaches that combine high spatial resolution, with the highest sensitivity (microfluorimetry) and a molecular fingerprint (surface-enhanced Raman spectroscopy - SERS) [2,3]. To gain an insight into the paint formulations, we included fibre optic reflectance spectroscopy in the visible (FORS) and Fourier-transform infrared spectroscopy (microFTIR). In situ techniques, such as microfluorimetry and FORS, can only be used for an in-depth analysis if supported by a made-to-measure reference database such as the one we have been building, over the past 15 years, based on studying and reconstructing the medieval processes for making pigments and paints that were used to create medieval manuscript illuminations [14-16].

To the best of our knowledge, in only three publications organic colorants were unequivocally identified in Islamic manuscripts; indigo was detected by microRaman and HPLC-DAD by Lucia Burgio (and co-workers) [17] and Teresa Espejo Arias (and co-workers) [18], respectively; carminic acid was characterized by surface enhanced Raman spectroscopy (SERS), by A. El Bakkali and co-workers [19]. The other publications rely essentially on energy dispersive X-ray fluorescence (EDXRF) and FORS that will give an indication of the possible colorants present; in the majority of the cases authors have proposed the use of "cochineal" [20- 23], and in one publication of "kermes" [21], **Table 5.1**. With FORS it is possible to discriminate between families of chromophores such as flavonoids and anthraquinones;

[77] Manuscript 19 is described as an oftalmological study, with 78 notes from the author itself which are a reflection about medical matters and a documental source for the study of both climate conditions and the population census of the Niger.

for the latter, it is also possible do distinguish between anthraquinone rings bearing or not bearing a carboxylate group. This is relevant because it allows to differentiate between anthraquinone chromophores produced by parasitic insects (e.g., carminic, kermesic and laccaic acids) from anthraquinones of vegetal origin, **Figure 5. 1** and **Figure 5.2**. The main results and analytical techniques for the study of organic colorants in medieval Islamic manuscripts are summarized in **Table 5.1** [17-28]. Another source of information on the dyes used are medieval technical texts and textile studies in the Islamic world, which show that the main colorants used to produce reds are anthraquinone and brazilwood based [29, 30].

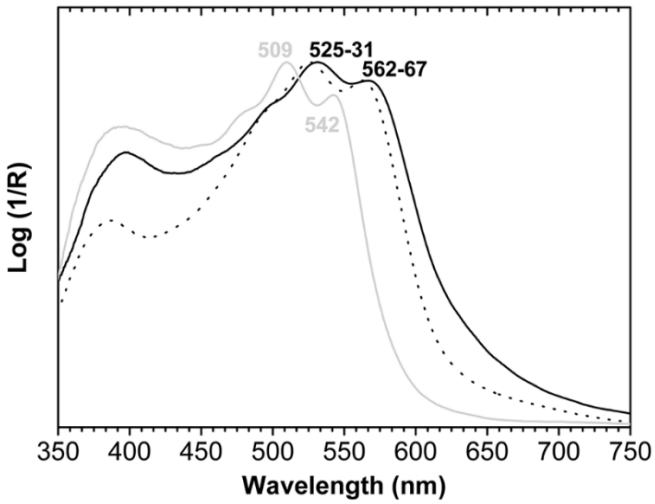

Figure 5. 1. Apparent absorbance spectra for anthraquinone lake pigments obtained from (grey) madder, *Rubia tinctorum* roots; (black) lac dye, *Kerria lacca*; (dashed) cochineal, *Dactylopius coccus*, applied as paints; the first and latter with arabic gum in paper, and the *Kerria lacca* with egg white in parchment.

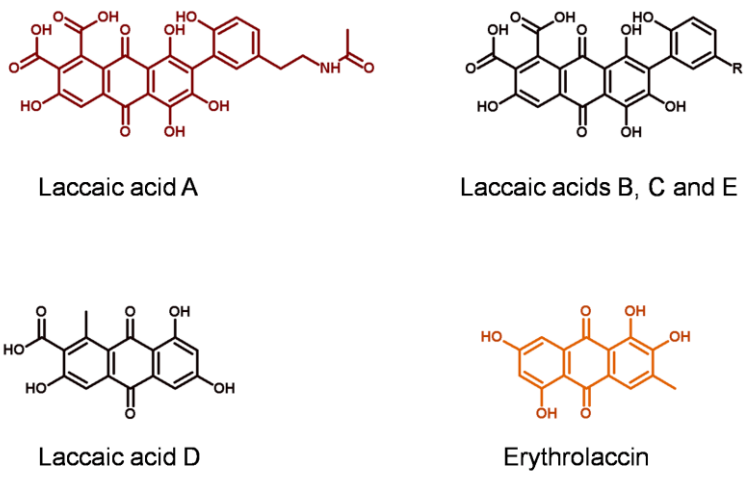

Figure 5.2. Chemical structures of laccaic acid A, laccaic acids B, C and E (B, R=CH_2CH_2OH; C, R=CH_2CHNH_2COOH; E, R=$CH_2CH_2NH_2$), laccaic acid D and erythrolaccin. Laccaic acid A is the main chromophore extracted from sticklac and displays a red to carmine color; erythrolaccin is yellow and is usually found in the resin.

Table 5.1. Organic dyes identified in medieval illuminated Islamic manuscripts, with information on the date of the manuscript, analytical technique used for identification and spectral data.

Dyes[§]	Manuscript date	Analytical technique	Spectral data	References
Cochineal	12th - 15th c.	μ-XRF; DRS	n.a.	Deroche, F. *et al*., 2005 [20]
Cochineal, Kermes	9th - 11th c.	DRS; μ-XRF; MO	n.a.	Roger-Puyo, P. *et al*., 2015 [21]
Not found	10th - 11th c.	μ-XRF; μ-Raman	n.a.	Hamdan, N. M., *et al.*, 2012 [22]
Cochineal	10th - 19th c.	DRS; MO; μ-XRF	λ_{abs}: 500,525,561 nm [#]	Roger, P. *et al.*, 2004 [23]
Not found	14th c.	μ-XRF; μ-Raman	n.a.	Duran, A. *et al*., 2011 [24]
Not found	14th - 16th c.	n.a.	n.a.	Beny, A. *et al.*, 2015 [25]
Organic red	16th - 18th c.	μ-Raman; SEM-EDX	SEM-EDX: Ca, Al, K, S	Tanevska, V. *et al.*, 2014 [26]
Not found	n.a.	MO; μ-XRF; Chromatography	n.a.	Arias, T., 1997 [27]
Not found	n.a.	MO; μ-XRF; μ-Raman	n.a.	Biddle, M., 2011 [28]
Cochineal	n.a.	μ-Raman; SERS; DRS	λ_{abs}: 500,528,570 nm SERS: 1582,1444, 1330, 1223 e 482 cm^{-1} [#]	El Bakkali, A., *et al.*, 2012 [19]
Índigo	16th - 18th c.	μ-XRF; μ-Raman	μ-Raman: 546,599, 1575, 1584 cm^{-1} [#]	Burgio, L. *et al*., 2008 [17]
Indigo	11th - 17th c.	DRX; SEM/EDX; μ- FTIR; HPLC;	HPLC: indigotin	Arias, T. *et al*., 2008 [18]

§ - Names given by the authors.
# - Data collected from the spectra displayed in the publications (not described in the text).

5.2. Materials and methods

5.2.1. The manuscripts and overall modus operandi

This project encompasses the study of five manuscripts, specifically a Koran, Mss 19, Al-Sarishi Poems, Prophet's Biography and Theology Treatise. The dating and typology of the manuscripts were defined by the president of the *Fondo Ka'ti*, Ismael Diadié Haïdara. All the details on the manuscripts can be found on **Table A4.2**.

The first screening of the manuscripts was carried out by FORS and microEDXRF. For each of the five manuscripts 3 folios were analyzed, except for the Koran and Mss 19, with 4 and 6 folios analyzed respectively, and each color was analyzed in three areas with three points per area per folio: Koran (fols 1, 30, 47 and 70), Mss 19 (fols 18, 19, 109, 110, 217 and 218), Prophet's Biography (fols 1, 4 and 107), Al-Sarishi Poems (fols 1, 4 and 10) and Theology Treatise (fols 17, 43 and 70). This allowed to select areas to be analyzed by microRaman and sampled for infrared spectroscopy (microFTIR) allowing us to characterize pigments, binders and gain insight into the full paint formulation. One or two areas per color per manuscript were selected for microsampling, except for the Al-Sarishi Poems were only the vermilion red was sampled, due to the fragile condition of the support. MicroRaman was used in situ, with 3 points per color per folio, or in microsamples. Microspectrofluorimetry was then used for both in situ and microsample analysis to identify the colorants present. The criteria for the selection of the folios for analysis was based on ensuring that all the colors present in the manuscripts were part of the selected folios and that they encompassed as much variability as possible.

5.2.2. Historically accurate reconstructions

The historically accurate reconstructions of lac dye have been prepared within the scope of a doctoral thesis, and more details can be found in previous publications in which these reproductions have been described [31-38]. For lac dye, fourteen recipes were selected from eight treatises/ recipe books: Ibn Bādīs manuscript (c. 1025) [33], Mappae clavicula (9th - 12th c.) [34], *Livro de como se fazem as cores* ('book of all color paints', 15th c.) [35], the Le Begue manuscript (1431) [36], the Bolognese manuscript (15th c.) [36], the Strasbourg manuscript (15th century) [37], the Montpellier manuscript (15th c.) [38] and the Paduan manuscript (late 16th to 17th c.) [36]. The main steps for the reproduction of lac dye lake pigments are common to all recipes. The resin, sticklac, is ground and a basic solution is added and then is filtered to remove impurities of the lac and its residual resin. Some recipes are complete at this point and do not add a complexing agent as a fourth step. In other recipes, Al^{3+} in the form of alum is added, to precipitate the colorant. In this work three recipes of lac dye will be used for comparison with the Islamic organic reds: chapter 6 ('*red ruby from the lukk'*) from the Ibn Bādīs manuscript, recipe 113 from the Paduan manuscript and chapter 13 ('*para fazeres nobre karmen'*) of the 'Book of all color paints'. For all three recipes the lac is added to water and no complexing agent is present. For the first, the recipe for red ruby of Ibn Bādīs, borax and sodium carbonate from ashes, are also added; for recipe 113 of the Paduan manuscript, tartar is added at the beginning; and for chapter 13 of the 'Book of all color paints', the extraction is carried out with urine and quicklime and ashes are then added. For a detailed description please see [31] and **Appendix A.1.1** for the transcription of the recipes.

5.2.3. Equipment

Micro-sampling

Micro-sampling of the manuscripts was performed with a microchisel from Ted Pella microtools under a Leica KL 1500 LCD microscope, (7.1x to 115x objective) and a Leica Digilux digital camera, with external illumination via optical fibers. Micro-samples were taken under a microscope, typically of 20–50 μm in diameter and as such invisible to the naked eye; as we have not yet obtained their weight, even though micro-scales have been used, we can use its detection limit to conclude that they weigh less than 0.1 μg.

Energy dispersive X-ray fluorescence (microEDXRF)

MicroEDXRF results were obtained using an ArtTAX spectrometer of Intax GmbH, with a low-power molybdenum (Mo) X-Ray tube attaining a microspot with a spatial resolution of circa 70 μm, an X-flash detector refrigerated by the Peltier effect (Sidrift), sustained by a mobile arm (providing a major freedom in choosing the spot of analysis). The accuracy of the incident beam position on the sample is achieved through three beams crossing diodes controlled by an integrated CCD camera; the characteristic X-rays emitted by the sample (at 40º) are detected by a silicon drift electro-thermally cooled detector with a resolution of 160eV at Mn-Kα. This apparatus allows for a simultaneous multi-element analysis in the element range from Mg (magnesium, atomic number 12) to U (uranium, atomic number 92). The experimental parameters used were: 40kV of voltage, 300μA of intensity, for 120s, under Helium gas flux. Si, Mn, Cu and Pb standards were used as calibration standards in the beginning and at the end of each day of data acquisition.

Fiber optic reflectance spectroscopy

The reflectance spectra were obtained with a reflectance spectrophotometer Ocean Optics, MAYA 2000 Pro, with single beam optical fibres, equipped with a linear silicon CCD detector Hamamatsu, with a spectral range of 200-1060 nm. The light source is a halogen lamp Ocean Optics HL-2000-HP, 20 W output, with a spectral range of 360-2400 nm. The analyses were obtained with 8 ms integration time, 15 scans, 8 box width, and acquired at 45º/45º (light source/acquisition), with a spatial resolution of 2mm. To calibrate the equipment a white reference was used, Spectralon® standard. The spectra were acquired in reflectance mode and presented as apparent absorbance A'=log10(1/R).

Microspectrofluorimetry

Fluorescence excitation and emission spectra were recorded with a Jobin–Yvon/Horiba SPEX Fluorog 3-2.2 spectrofluorometer hyphenated to an Olympus BX51M confocal microscope, with spatial resolution controlled by a multiple-pinhole turret, corresponding to a minimum 2 μm and maximum 60 μm spot, with 50× objective. Beam-splitting is obtained with standard dichroic filters mounted at 45°; they are located in a two-place filter holder. For a dichroic filter of 525 nm, excitation may be carried out until about 510–515 nm and emission collected after about 530–535 nm ("excite bellow, collect above" principle).The optimization of the signal was performed daily for all pinhole apertures through mirror alignment, following the manufacturer's instructions, using a rhodamine standard (or other adequate reference). Standard dichroic filters of 525 and 600 nm were used where the emission spectra were acquired exciting at 515 nm and excitation spectra were recorded collecting the signal at 610 nm. This enables both the emission and excitation spectra to be collected with the same filter holder. A continuous 450 W xenon lamp, providing an intense broad spectrum from the UV to near-IR, is directed into a double-grating monochromator, and spectra are collected after focusing on the sample (eye view) followed by signal intensity optimization (detector reading). The pinhole aperture that controls the area of analysis is selected based on the signal-to-noise ratio. Usually, for weak to medium emitters, it is set to 8 μm (pinhole 5); in this work for very weak signals 30 μm spot was also used (pinhole 8) with the following slits set:emission slits = 3/3/3 mm and excitation slits = 5/3/0.8 mm. Emission and excitation spectra were acquired on the same spot whenever possible. For more information please see [10, 11].

Fourier transform infrared microspectroscopy (microFTIR)

Infrared analyses were performed using a Nicolet Nexus spectrophotometer coupled to a Continuμm microscope (15x objective) with a MCT-A detector cooled by liquid nitrogen. The spectra were collected in transmission mode, in 50 μm areas resolution setting 4 or 8 cm^{-1} and 128 scans, using a Thermo diamond anvil compression cell. For some infrared spectra the system was purged with nitrogen prior to the data acquisition; for all infrared spectra the CO_2 absorption at circa 2400-2300 cm^{-1} was removed from the acquired spectra (4000 - 650 cm^{-1}). To improve result robustness, more than one spectrum was acquired from different sample spots.

5.2.4. Chemometrics analysis based on excitation spectra

The data from the case studies were compared with databases for red lake pigments resourcing to a chemometric analysis method [4]. The first database is built up from historically accurate reproductions composed of brazilwood, cochineal, lac dye, and kermes. For the second approach, the database is built up of data from artworks from medieval manuscripts to textiles (11^{th} – 15^{th} c.) where lac dye, cochineal and brazilwood were identified. The chemometric analysis was based on Hierarchical Cluster

Analysis (HCA) resourcing to the Ward's algorithm and the Mahalanobis distance. For the first database, the HCA method was fed with scores resulting from a principal component analysis of the dataset. For the second, the HCA algorithm was fed with spectral data (not requiring a previous PCA step). In both cases, excitation spectra were preprocessed by applying the Haar transform followed by the 1st derivative (2nd order). Normalization by area (unit area) is also typically used for the analysis of fluorescence data and was also considered and applied subsequently to the first two methods.

5.3. Results and discussion

In a first observation, manuscripts' support displayed a reduced pliability and low mechanical strength. No extensive paint loss was observed, except for the Koran, where we found only traces of the original colors (**Figure A4.1** of **Appendix A.4**). In the red colors that will be discussed, EDXRF analysis detected only the chemical elements of the paper support, indicating the presence of organic chromophores. The organic reds in all 5 manuscripts studied are perceived as dark reds, **Figure 5.3**. When used for writing and drawing, in each manuscript, no distinction was found.

Figure 5.3. Details of the red colorants used in the drawings (*top*) and writing inks (*bottom*), in the illuminated manuscripts studied. Usually based on lac dye, except for the following case studies; vermilion and an iron-gall based chromophore were detected in the writing ink used in Mss 19 (*2nd detail, bottom*); in the Theology Treatise an organic purple was used in the drawing ink (*3rd detail, top*).

5.3.1. Characterization of the main chromophores

Microspectrofluorimetry in the visible

The emission and excitation spectra for the dark red inks/paints are plotted in **Figure 5.4**, together with the best matches we obtained with historical medieval paint reproductions. In all cases the best match was obtained with a lac dye based color; for Al-Sarishi Poems, Prophet's Biography and Koran, the match with the database is very good, enabling a safe assignment of the presence of a lac dye red.

In the Koran, generally, we obtained spectra with very low intensity, and it is important to remember that in this document we do not find intact paints, but traces, as seen in **Figure A4.1** of **Appendix A.4**. In all excitation spectra, the presence of the shellac resin was detected through erythrolaccin (max at 474 nm) [3], and a good match with Ibn Bādīs red was obtained [31, 32, 38], **Figure 5.4**. For the emission spectra, we found both the spectral envelope depicted in **Figure 5.4** as well as another emission signature with maxima at 560 nm and a shoulder at 585 nm (fol 30r, **Figure A4.2** of **Appendix A.4**). In both cases, the match of Ibn Bādīs red colorants with the emission spectrum representative of the red chromophore present in the Koran is not satisfactory.

Characterized by a maximum at 592 nm and a shoulder at 607 nm, the emission spectrum of Mss 19 compares well with what is described for the reds in the Koran, and therefore the closest match is again with the reproduction from the treatise of Ibn Bādīs.

The reds used in Al-Sarishi Poems and Prophet's Biography display similar emission and excitation spectra and for that reason, in **Figure 5.4**, we only represent the spectra for Al-Sarishi Poems (emission maxima found at 582 and 604 nm; and excitation at 517 and 551 nm). The spectra obtained agree very well with the reconstruction described in the Paduan manuscript as recipe 113 [31-33], characterized by a L*=53, a*=24 and b*=7; i.e., a red color with a small component in the yellow. These are perceived almost as carmines and are lighter than the dark reds from the other manuscripts that will be next described, **Figure 5.3**.

The colorant found in the Theology Treatise is characterized by an emission and excitation spectra shifted to lower wavelengths (emission and excitation maxima at 583 nm and 532 nm, respectively) when compared to Al-Sarishi Poems. In this case, it was not possible to find a reference that could match closely with both spectra; one of the best matches obtained was with the carmine color produced following the recipe 113 from Paduan manuscript.

The low intensities obtained in the excitation spectra of the dark red colors indicate that these colors are not based in Al^{3+} complexes (when this is the case, we observe a tenfold increase in the signal intensity), or, if present, they do not represent the major chromophore. Overall, the matches with Ibn Bādīs red ruby and Paduan 113 confirm these conclusions as these colors were prepared without the addition of alum.

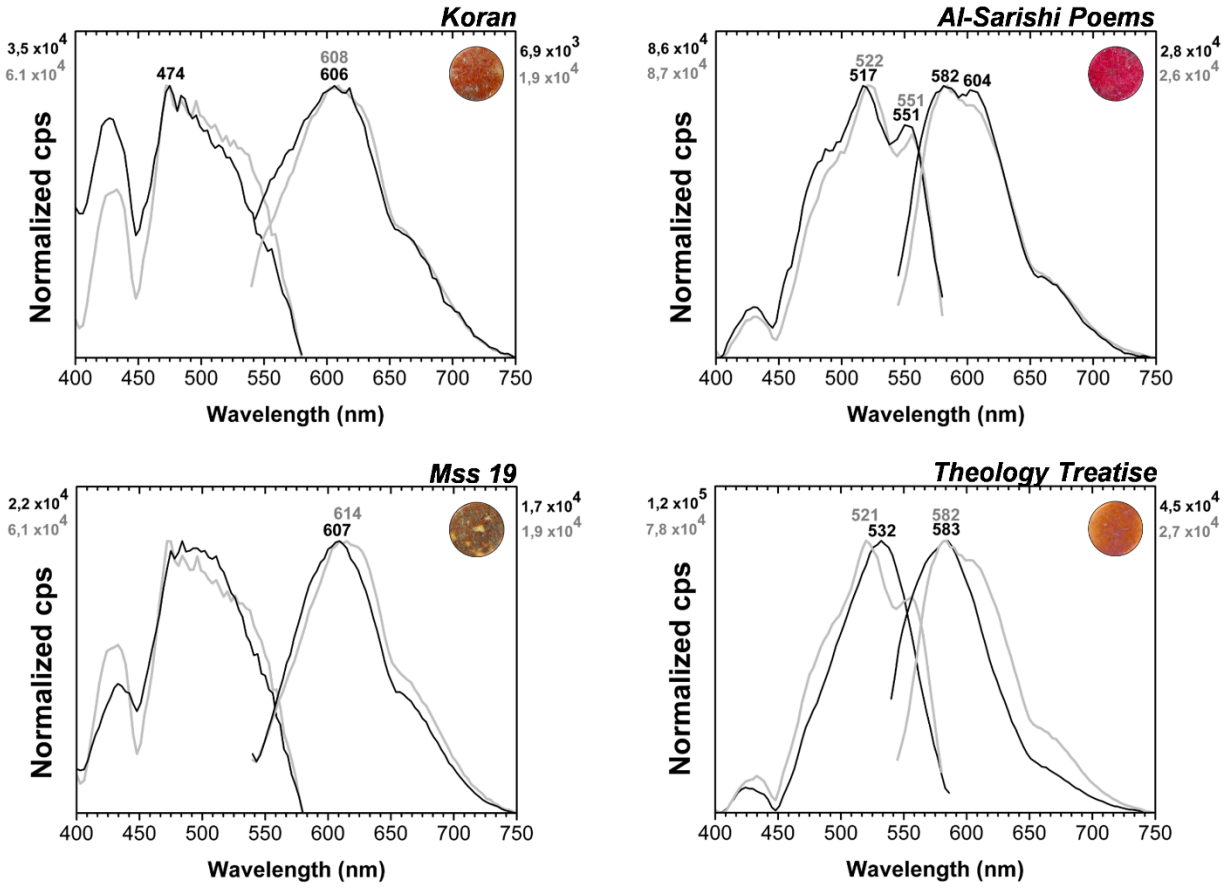

Figure 5.4. Excitation and emission spectra for the organic red colorants in Islamic manuscripts (*black*), compared with historically accurate reconstructions of lac dye paints (*grey*). For the Koran and Mss 19 with recipe for *red ruby* of Ibn Bādīs; for Al-Sarishi Poems and the Theology Treatise (as well as for Prophet's Biography, not shown) with recipe 113 of Paduan manuscript. For more details please see text.

FORS in the visible

Generally, the reflectance spectra acquired match the same paint reproductions as the fluorescence spectra, as follows: the spectrum of the reds of Koran and Mss 19 display a satisfactory correlation with recipe for Ibn Bādīs' *red ruby*; Al-Sarishi Poems and Prophet's Biography with recipe 113 from the Paduan manuscript **Figure 5.5**.

On the other hand, the Theology Treatise displays a better match with chapter 13 of the 'book of all color paints' and not with 113 from Paduan manuscript as was the case with emission and excitation spectra [31-34]. In these spectra, the first band is attributed to the reflectance from the support, at 400 nm for paper and 375 nm for parchment; possibly due to the lower thickness of the original colors when compared with our reproductions (reflectance spectra for parchment and paper are found in **Figure A4.3** of **Appendix A.4**). Also, the chromophore is not as well defined as in the reproductions, **Figure 5.5**.

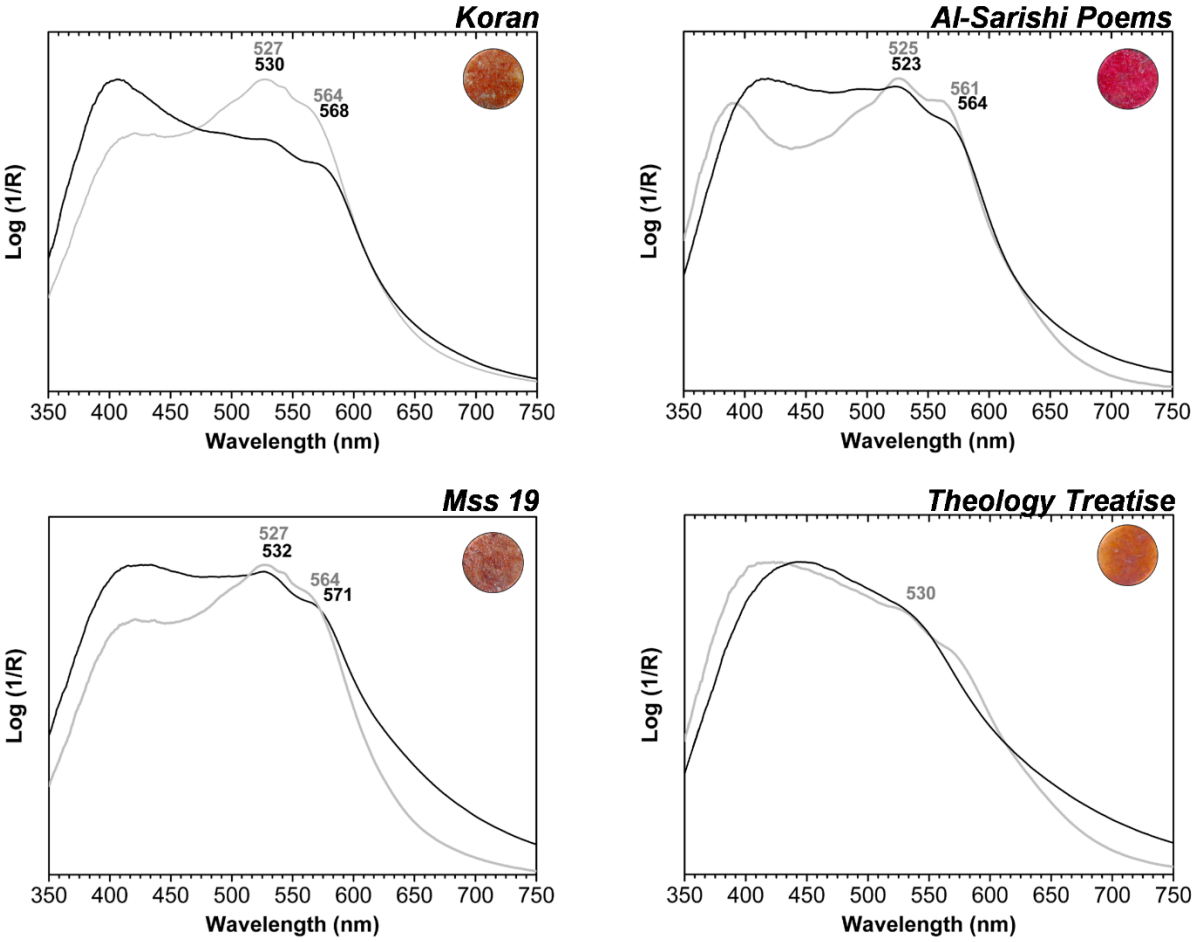

Figure 5.5. Apparent absorption spectra for the organic red colorants found in Islamic manuscripts (*black*), compared with historically accurate reconstructions of lac dye (*grey*). For the Koran and Mss with an Ibn Bādīs paint for *red ruby*; for Al-Sarishi Poems with recipe 113 of the Paduan manuscript; and for the Theology Treatise with chapter 13 of the 'book of all color paints'.

Chemometrics based on microspectrofluorimetry data

A chemometric model was applied to verify if additional information could be extracted from the fluorescence spectra. The analyzed samples were tested against two databases of spectra from (*i*) historically accurate reconstructions of lac dye, brazilwood, cochineal and kermes and from (*ii*) historical samples dated from 11th – 15th centuries [4]. An unsupervised modelling was chosen, hierarchical cluster analysis (HCA). For the comparison with samples from the first database, the HCA method was fed with five principal components. These principal components were generated from a previous PCA model. In agreement with the microspectrofluorimetry analysis, generally, all the organic reds were clustered as lac dye. A more detailed analysis shows that the Prophet's Biography, Al-Sarishi Poems and Mss 19 are also close to the kermes cluster. The Theology Treatise, however, was more difficult to predict, and

although it falls within the lac dye cluster, it alters the model greatly. These results show that for the Theology Treatise the model could not add any relevant information. See **Appendix A.4**.

Another approach was attempted, now comparing the testing samples against a database of historical samples, artworks from medieval manuscripts to textiles (11th – 15th c.) where lac dye, cochineal or brazilwood were present as the main chromophores. With this approach the results were substantially improved. With this database it was found that better results arose without the use of PCA, so the HCA algorithm was fed directly with pre-processed spectra. Considering the excitation spectra dataset alone, the HCA method revealed a successful clustering of the colorants, as seen in **Figure 5.6**. The model considers the data from the Theology Treatise, the Al-Sarishi Poems and the Prophet's Biography within the lac dye cluster, similar to the lac dye reds used in the *Book of Birds* (1183-4) produced in the *scriptoria* of the monastery of Lorvão (Lorvão 5). The dark reds in this manuscript are characterized by an excitation maximum at 525 nm with shoulders at 473 nm and 553 nm, and emission maximum at 587 nm; which compares well with the excitation maximum at 517 nm with a shoulder at 551 nm, and emission maximum at 582 nm for the red colors in the Al-Sarishi Poems and the Prophet's Biography, see **Figure 5.4**. Possibly due to the presence of shellac resin found in the Theology Treatise, the model predicts it similar to the *Book of Birds*.

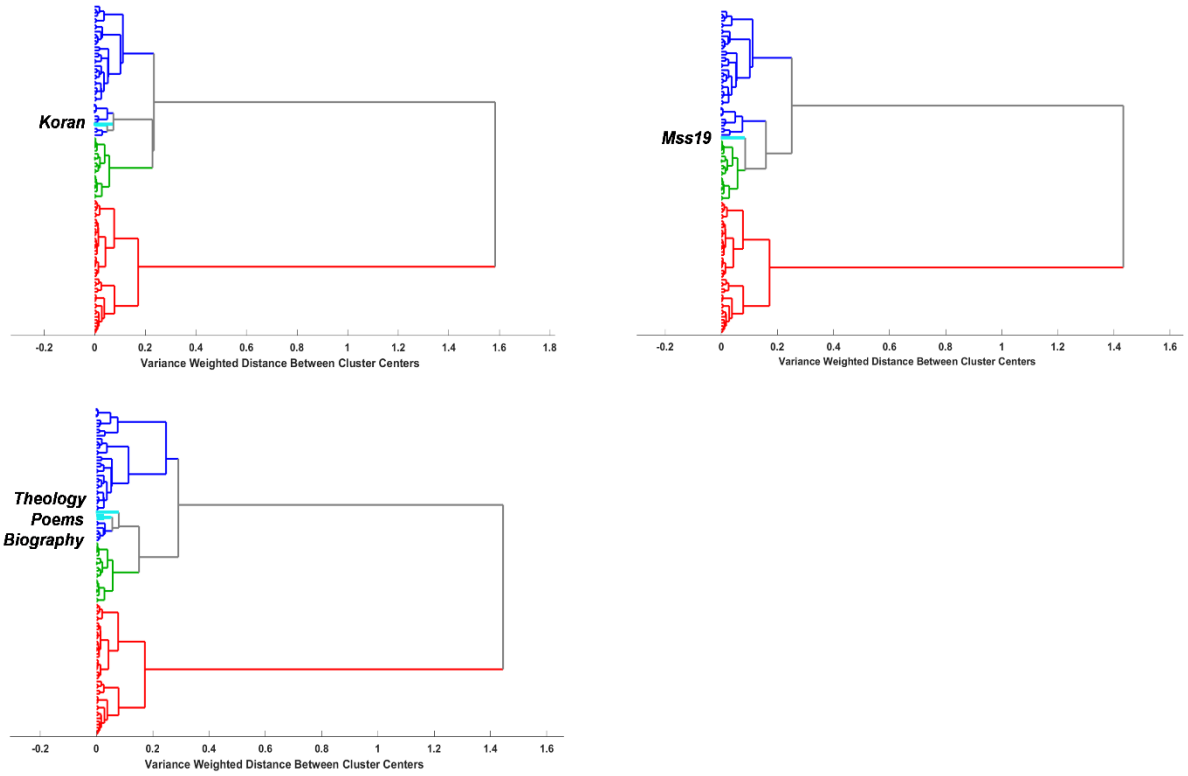

Figure 5.6. Dendrogram generated by HCA applied to excitation spectra using a model composed of data acquired from artworks (lac dye (*blue*), cochineal (*green*) and brazilwood (*red*)). The data from the Islamic manuscripts was predicted within the model (*light blue*).

The Koran' colorants are also found in the lac dye cluster, sharing similarities with the manuscript Lorvão 13 (13th century), with excitation maxima at 472 nm and emission at 591 nm.

For the Mss 19, however, it appears that the model locates this sample between the lac dye and the cochineal clusters, unable to place it with certainty within a colorant class.

As an overview, in **Figure 5.7**, we represent the spectra for the organic red colorants found in Islamic manuscripts compared with real case studies of medieval Portuguese manuscripts. As expected by the fluorimetry discussion, where the low intensities obtained in the excitation spectra indicate that these colors do not present Al^{3+} complexes as the main chromophores, also in this model all the samples were predicted within a cluster of lac dye reds in which Al^{3+} is absent or present in very low amounts.

At this point, these results reveal that other molecular fingerprint techniques, such as surface-enhanced Raman spectroscopy, should be used to confirm the identification of the red colorants present in Theology Treatise.

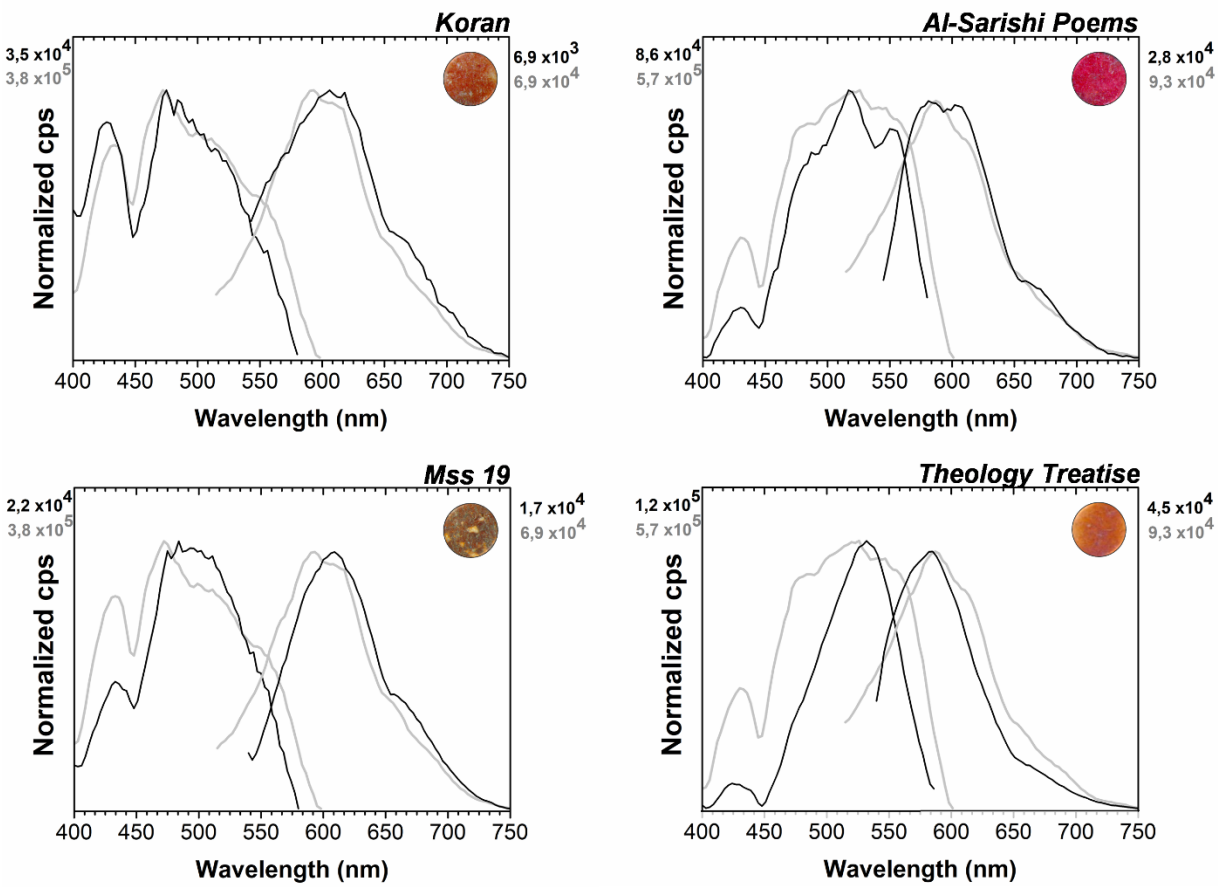

Figure 5.7. Excitation and emission spectra for the organic red colorants found in Islamic manuscripts (*black*), compared with case studies of medieval Portuguese manuscripts, from monastery of Lorvão, (*grey*). For the Koran and Mss 19 with the *Lectionarium Temporale*, Lorvão 13; for Al-Sarishi Poems and the Theology Treatise the comparison is made with the *Book of birds*, Lorvão 5.

5.3.2. Characterization of the paints

Binding media characterization by infrared spectroscopy

The discussion of the binders used is more complex and will be carried out by comparison with original samples from Portuguese monastic collections (12th-13th c.), because we studied it in-depth and the number of spectra acquired makes it a robust dataset [4]. In the *scriptoria* of these three important Portuguese monasteries, a variety of lac dye red paints were prepared; e.g. fillers could be or not present (calcium carbonate or/and gypsum), different proportions of shellac resin and proteinaceous binder have been found [1, 3, 31].

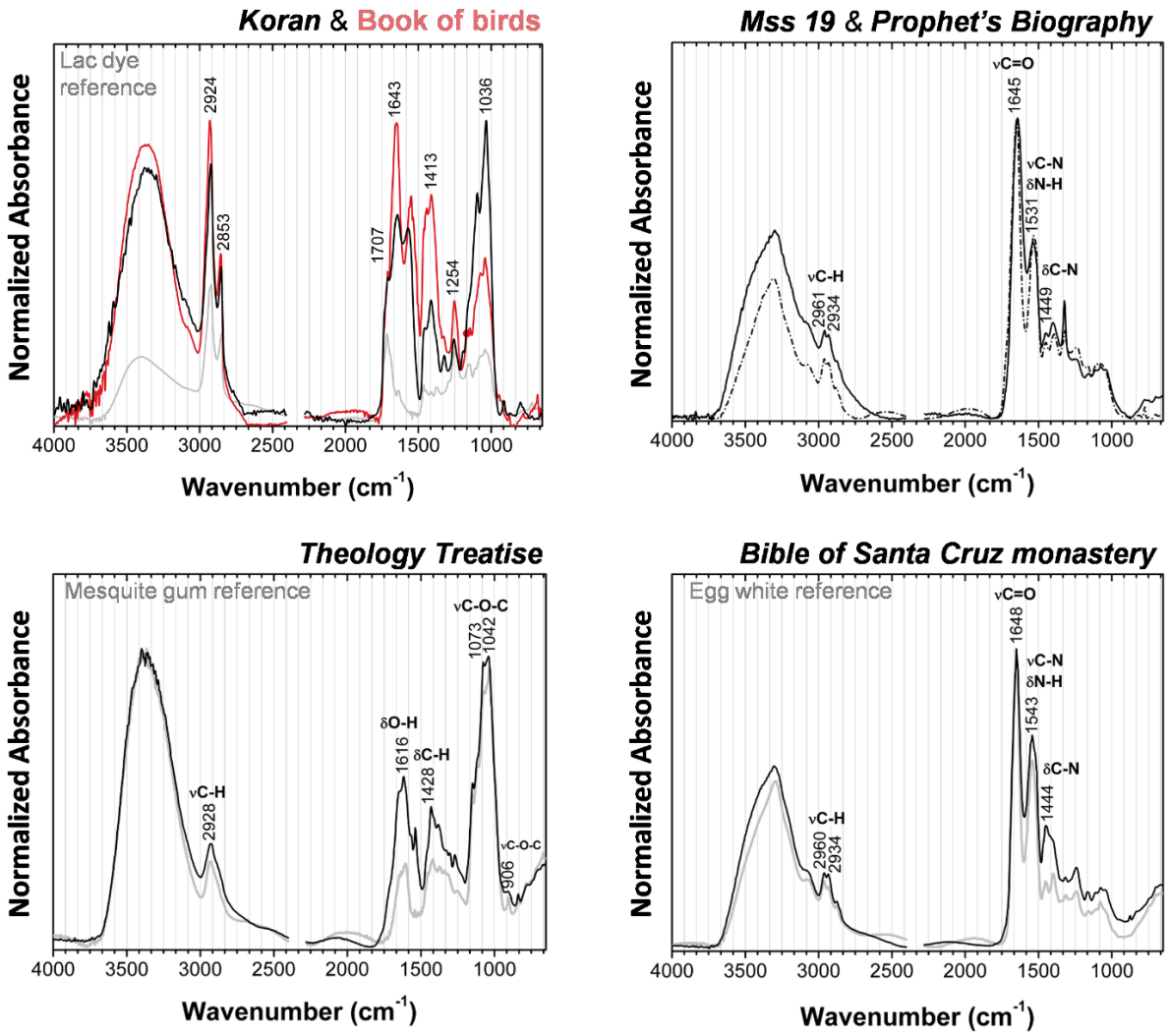

Figure 5.8. Infrared spectra for the organic red colorants found in Islamic manuscripts: *from up to bottom, left to right*, Koran (*black*) with the *Book of birds* from the monastery of Lorvão (*red*) and lac dye reference (*grey*) ; comparison between the Mss 19 (*black*) and the Prophet's Biography (*grey*); Theology Treatise (*black*) with mesquite gum reference (*grey*); and *Bible* of the monastery of Santa Cruz of Coimbra (*black*) with egg white reference (*grey*).

The infrared spectra of the red colorants in the Koran, Theology Treatise, Prophet's Biography and Mss 19 are represented in **Figure 5.8**. The presence of the C-H stretching at 2924 and 2853 cm^{-1} is very clear in all Koran infrared spectra, confirming unequivocally the presence of shellac resin as anticipated by the fluorescence spectrum. A very good match was found with a lac dye red used to paint the *Dove diagram* in Lorvão' *Book of birds*, **Figure 5.8**. The shellac resin spectral envelop in the fingerprint region also compare well with a shellac reference. Overall, the two medieval paint spectra match well, and the main differences observed are in the amount of proteinaceous binder, higher in the Portuguese color.

A clear proteinaceous fingerprint is observed in the spectra from red paints in the Prophet's Biography and Mss 19, but the presence of shellac resin could not be detected, possibly because if falls below the detection limits of this technqiue (<10%). For these samples a very good match was found with the carmine color used to paint the initial in fol.1v of the monumental *Bible of the Holly Cross monastery*, Santa Cruz 1 (except for the presence of calcium carbonate, present in low amounts in these Portuguese paints, but not detected in the Islamic manuscripts), **Figure 5.8**. On the other hand, in the Theology Treatise, a perfect match was found with a polysaccharide such as mesquite gum, **Figure 5.8**.

Assessing degradation by infrared spectroscopy

Calcium oxalate was detected in most of the samples (through the sharp bands at 1625 and 1323 cm^{-1}). It was present in higher amounts in Koran and Mss 19 dark reds. Nati Salvadó and co-workers, proposed that the presence of calcium oxalate, as weddellite (dihydrate form) and/or whewellite (monohydrate) in Cataluña medieval paints, may be ascribed to the binding media degradation [39]. Considering the small number of microsamples that we studied in this work, this cannot be used to conclude that in these manuscripts carmine colors display a higher level of degradation. However, we think it is possible to conclude that overall they display more binder degradation than what was found in 12^{th}-13^{th} c. Portuguese carmine paints.

In fol. 30r of the Koran, in the dark red color, we detected for the first time the presence of a soap, which we assign to calcium palmitate by comparison with its C-H stretching at 2916 and 2850 cm^{-1} as well as carboxylate bands at 1574 and 1540 cm^{-1}, **Figure 5.9** [40].

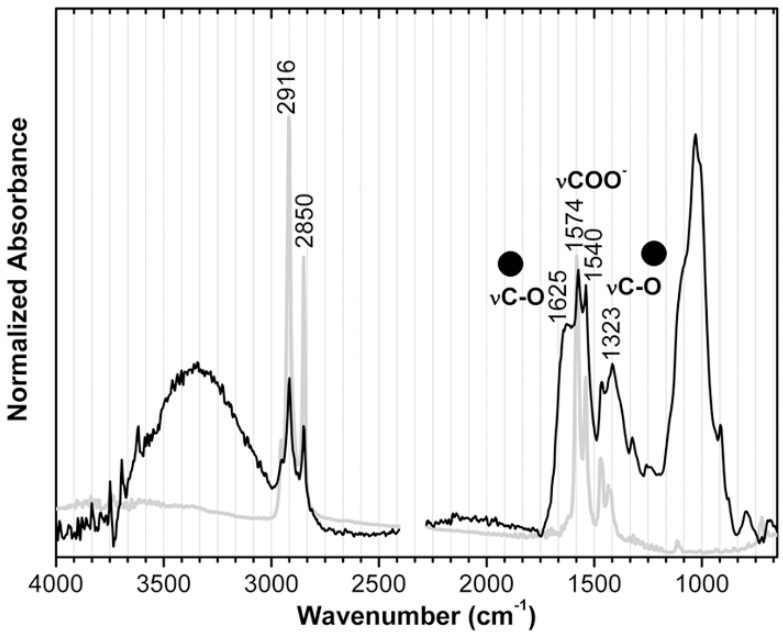

Figure 5.9. Infrared spectra for the organic red colorant found in the Koran, fol. 30r (*black*), with reference of calcium palmitate (*grey*) and fingerprint of calcium oxalate (●).

5.4. Conclusions

For the first time the use of lac dye in Islamic manuscripts was unequivocally proved. For the red inks used in the Koran, Al-Sarishi Poems and Prophet's Biography it was possible to further ascribe a specific recipe to produce the colorant and to disclose the paint formulation. In the Koran, lac dye cannot be present as a Al^{3+} complex, and in Al-Sarishi Poems and Prophet's Biography our data indicate that possibly Al^{3+} complex cannot represent the main chromophore. Additives and binding medium further disclose the richness and specificities of these formulations: all lac dye based, but all different. Chemometric analysis confirmed these assignments and further matched the colorants with data from Portuguese manuscript illuminations, the *Book of Birds* and *Lectionarium Temporale* from the monastery of Lorvão, Lorvão 5 and Lorvão 13, respectively.

In previous studies, for organic reds only "cochineal" or "kermes" have been identified. Importantly, with one exception [19], the identifications have been based on FORS-Vis data. For this reason, they must be considered with caution, as although this technique is able to distinguish between traditional anthraquinone dyes of animal or plant origin, **Figure 5. 1**, and to indicate the main chromophore present, it cannot assess its precise nature (kermesic, carminic or laccaic acids), in ancient manuscripts.

On the other hand, for the dark reds applied in the Theology Treatise, in situ methods supported by a robust database of medieval reconstructions could not provide an unequivocal molecular characterization of the main chromophore.

This study shows, for the first time, the presence of lac dye in islamic manuscripts, highlighting the richness and diversity of the paint formulations used. The very good spectral matches with both fluorescence emission & excitation spectra as well as with infrared data enable us to conclude that the chromophore is well preserved, but the binding media show signs of severe degradation. The presence of lac dye in these manuscripts agrees with the historical sources and the use of lac dye in the Arabic world [30].

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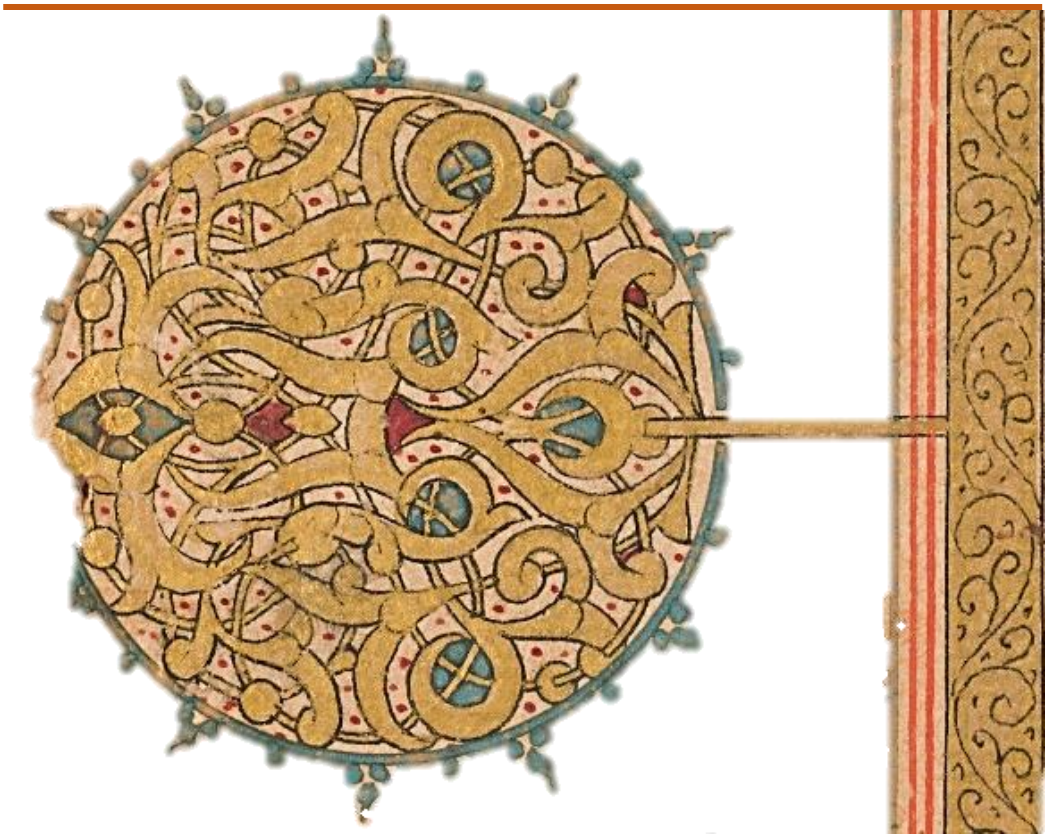
General conclusions and future research



Detail from the Biography of the Prophet, 1468
fol. 1 © Fondo Ka'ti

Chapter 6. General conclusions and future research

6.1. Discussion

In the past few years we have been particularly interested in the development of methodologies that will promote a complete characterization of the organic colorants used in the past. This doctoral project has demonstrated the ability of chemometrics applied to fluorimetry data to identify and characterize medieval lake pigments. The methodology developed enabled the in situ analysis of artworks, allying high selectivity and spatial resolution with the simultaneous acquisition of emission and excitation spectra, which facilitates a more accurate identification of natural organic colorants [1, 2]. Moreover, this analysis can be applied in situ in artworks, which can be transported to the lab, without the need of microsampling. The use of chemometrics to simplify the data analysis and to extract more information, which would have not been possible through the naked eye, allowed the in-depth analysis of color paints [3].

In **Chapter 2**, a robust database of historically accurate reconstructions has confirmed the potential for microspectrofluorimetry in the assessment of the chromophore's environment, i.e. the paint formulation. In **Chapter 3**, data acquired from artworks, analyzed and fully characterized by a multi-analytical approach developed by the team at DCR FCT NOVA, explored the intricacies of the formulations and the effectiveness of this methodology to explore similarities between color paints aged naturally.

The systematic studies done on medieval manuscripts, by researchers such as Ricciardi and Coupri, have been able to propose chronologies for the use of pigments [4, 5]. The last 15 years of investigations on the color materials in Portuguese monastic manuscripts and books of hours in Portuguese collections have led to the argument that organic colors are as important as pigments in establishing a chronology, particularly the red (pink, carmine and dark red) and purple hues and can provide important clues on the circumstances of the artwork's production. [6-9]. Therefore, the in-depth characterization of color paints, as provided by this doctoral project, enable a deeper understanding of the making of the colors and paint formulations present in historical artworks, enabling advances in art technological source research

The future development of a software with a search algorithm, based on the workflow is presented in **Figure 6.1**. The data from the unknown sample will be uploaded to the algorithm where it is subjected to the pre-processing required. The data is then added to the calibration set where a dendrogram is developed. To the user, it is given the values of proximity to the samples from the calibration set. This model is therefore dependent on the projected samples. Parallel to this model, the data after the pre-processing is also projected to the SIMCA model, being given to the user the percentage of certainty that the sample belongs to a specific class. This model is independent of the projected samples.

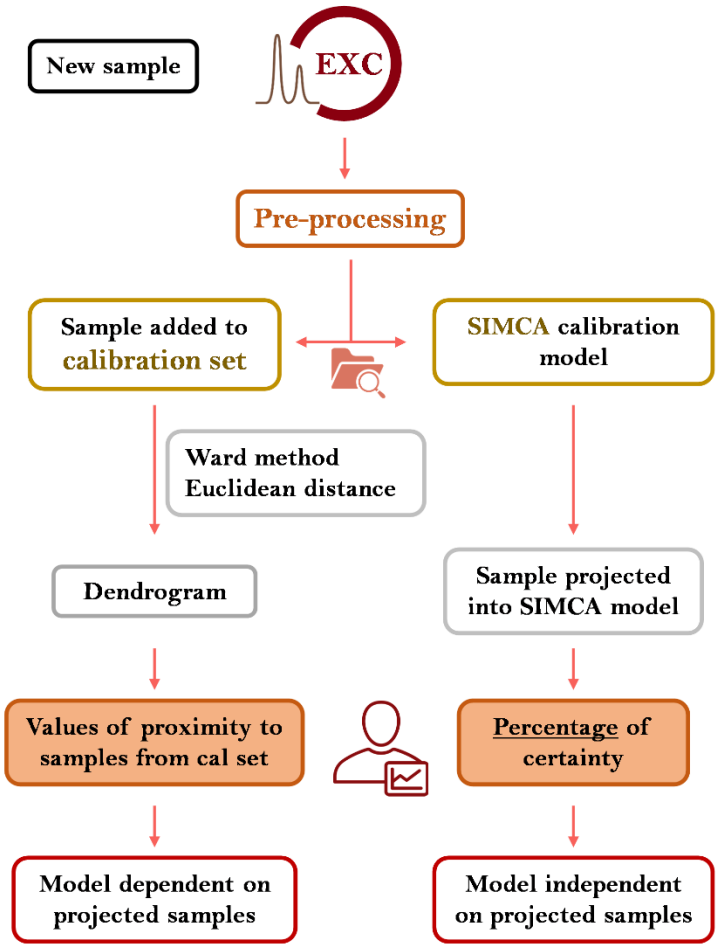


Figure 6.1. Workflow proposed for the development of the search algorithm.

This methodology was successfully applied on the case studies proposed for this project. For the **first**, the Ajuda Songbook, a complete molecular palette characterization allowed to propose a production date for this manuscript (end of the 13th, beginning of the 14th century). The presence of mosaic gold in this manuscript indicates a 14th century, while the use of orpiment yellow could push the date back into the 13th century. The methodology developed, allied with a multi-analytical approach, enabled the identification of brazilwood, which was also an important clue for the dating of the manuscript.

In the Middle Ages, Ceylon (Sri Lanka) was a center for exportation of brazilwood. It was sent to Europe through Alexandria in large amounts and in 1290, Marco Polo referred to the use of *Caesalpinia sappan* in the East as a dye. It became a significant item in the European commerce of the Middle Ages [10, 11] – so much so that Pastoureau refers to its use to dye textiles, being a fashionable color among the French aristocracy [12]. The use of brazilwood at a period before the discovery of Brazil points out to the exportation of brazilwood from Ceylon, and that the illuminator or the patron was knowledgeable of its use as a fashionable color among French aristocracy. The use of this color in the manuscript is not behind its European counterparts, on the contrary, it is, as far as we know, the first evidence of brazilwood applied to medieval manuscripts.

The chromatic palette used in the Ajuda Songbook demonstrates the desire and the resources to produce a luxurious manuscript. We do not have stylistically or thematically similar images to the Songbook's illuminations, nor do we have sufficient information to propose the existence of a *scriptorium* connected to the Portuguese court. We now plan to pursue new lines of research to achieve a better understanding of the use of brazilwood, yellow ochre and mosaic gold paints in medieval manuscripts. These future studies must also include additional comparative analyses of contemporary Iberian illuminated manuscripts and European songbooks, as well as the further analysis of pigments in Alfonsine codices, particularly the *Cantigas de Santa Maria* (*Códice de los Músicos* and *Códice Rico*).

The opportunity to study five Islamic manuscripts, possibly produced in Al-Andalus (12^{th} – 15^{th} century) presented as our **second case study**. The methodology developed and tested in this project allowed for a complete characterization of the red colorants applied on the manuscripts and the use of the artworks database was crucial for the characterization of these lakes. This filled a gap within scholarly knowledge regarding the use of red organic colorants in Islamic manuscripts. In previous studies, for organic reds only "cochineal" or "kermes" have been identified. Importantly, with one exception [13], the identifications have been based on FORS (Vis) data.

The similitudes of the lac dye identified with those found in Portuguese medieval illuminations is interesting, and in accordance with literature, since it is known that Al-Andalus would acquire materials from the commercial routes established by the Arabs [14, 15]. Although producing and exporting several materials, Andalusi dyers would turn to international trade for the acquisition of imported lac [15, 16]. By the 11^{th} and 12^{th} century, lac was one of the commodities traded in the Western Mediterranean [15, 16].

Moreover, the very good spectral matches with both fluorescence emission and excitation spectra as well as with infrared data enable us to conclude that the chromophore is well preserved, but the binding media show signs of severe degradation. The presence of oxalate compounds, possibly resulting from the binding media degradation, demonstrates the recent and dramatic history of these books. This characterization will allow for a more informed decision-making in the conservation process of these manuscripts. We can now pursue further studies of Islamic manuscripts and follow the use of this colorant in Medieval illuminations.

6.2. Future research

As a continuation of this work, in a project financed by the Portuguese Foundation for Science and Technology entitled "*Polyphenols in Art: chemistry and biology hand in hand with conservation of cultural heritage*" [78] an interdisciplinary team will gather to characterize the molecular structures of the writing inks and yellow paints, used in the medieval manuscript. Specialists from several areas of research have joined for this venture[79], collaborating in using chemistry for providing fundamental knowledge for these structures.

Iron gall inks are usually described as iron-polyphenol complex formed by the combination of iron salts to plant extracts, such as galls from the oak *Quercus infectoria*, to which a polysaccharide, such as gum arabic, was added see **Figure 6.2**. Nowadays, these materials pose a major conservation issue in heritage collections due to degradation. The models used by the scientific community to describe the iron complexes assume that gallic or tannic acid were available in the gall extracts to bind iron, which have been recently proved to be incorrect, and that gall extracts are characterized by a highly complex mixture of derivatives of gallic acid in the form of polygalloyl esters of glucose, also called gallotannins [17, 18]. This project envisages the characterization and understanding of these iron-polyphenolic complexes, relying on simple standards of these complexes, as well as historically accurate reconstructions of iron-gall inks. This will promote advances in polyphenolic research and better-informed conservation strategies.

Figure 6.2. Main steps and ingredients in the production of iron gall ink: an extract solution from galls, to which iron sulphate is added, forming a black precipitate. Gum arabic is added to keep the precipitate in suspension, adapted from [19].

[78] Project Polyphenols in Art, PTDC/QUI-OUT/29925/2017; coordination: Maria João Melo (PI) (DCR-LAQV/Requimte, FCT NOVA) and Natércia Teixeira (co-PI) (Requimte, FCUP).

[79] Specialists from the Faculty of Sciences and Technology (Cultural Heritage and Responsive Materials (CHARM) group from the Laboratório para Química Verde (LAQV) and the Department of Conservation and Restoration) from NOVA University, the Faculty of Sciences from the University of Oporto (Food Polyphenols Lab), Instituto Superior Técnico (Centro de Química Estrutural, CQE) and the Faculty of Sciences from the University of Lisbon (Centre for ecology, evolution and environmental changes, cE3c).

On the other hand, yellow dyes will also be approached in this project. Yellow dyes sources were probably gathered locally, and so the variety depends on geographical location, as well as chronology of use. As Dominique Cardon stated, "*if dyers, like artists, worked with a palette, they would need one dedicated entirely to the many yellows that can be obtained from plants that seem to have captured within themselves the very color of the sun*" [20]. In fact, yellow plants could have been more regional than any other source of organic colorants and their identification in artworks, such as medieval illuminations, poses an altogether different challenge due to their fading [21].

To assess their possible use, we will collect local plant species that could have been used as sources of yellow dyes in medieval times. The extract of these plants will be profiled by HPLC-DAD-HRMS which will enable an identification and a semi-quantification of the yellow polyphenols. This characterization backed on references of dyed textiles will allow to select the species which were possibly collected as a source of yellow dyes. After this first selection, reconstructions of medieval paints will be used to assess color saturation and stability. This later will be axed on quantum yields of reaction (obtained in homogeneous and heterogeneous media) and compared with known data from other well-characterized reference samples, e.g. brazilwood and cochineal. This photochemical study will enable another level of "triage" in assessing if any of the collected plants could have been a source for the "lost yellows" in medieval manuscripts, see **Figure 6.3**.

Finally, we envisage the construction of a database, and the application of new methodologies, such as that developed in this doctoral project, that will allow for the identification of yellow dyes in medieval manuscripts.

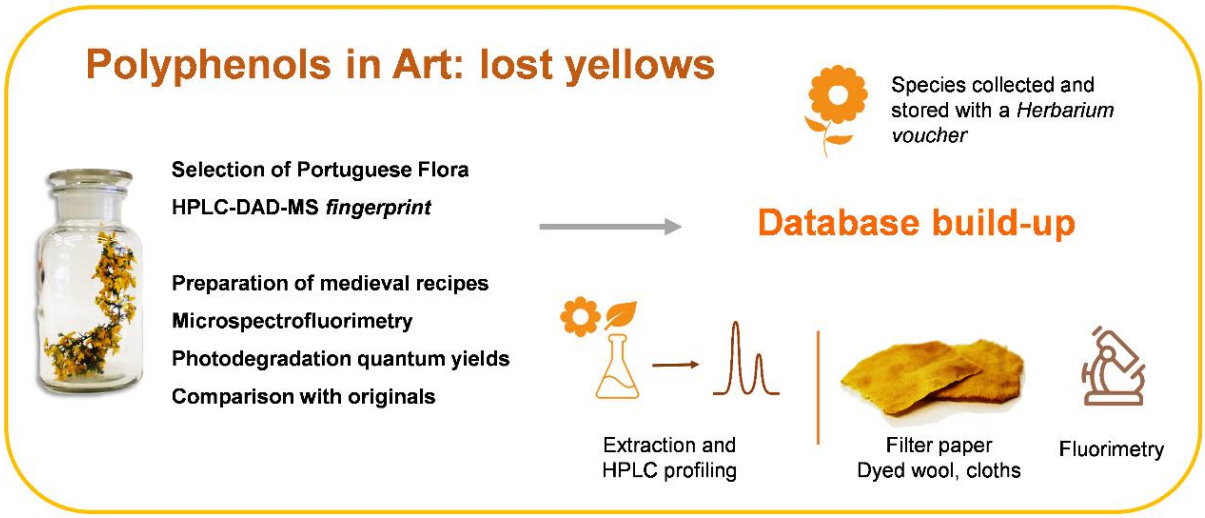


Figure 6.3. Project for the characterization of local plant species that could have been used as sources of yellow dyes in medieval times.

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Appendices

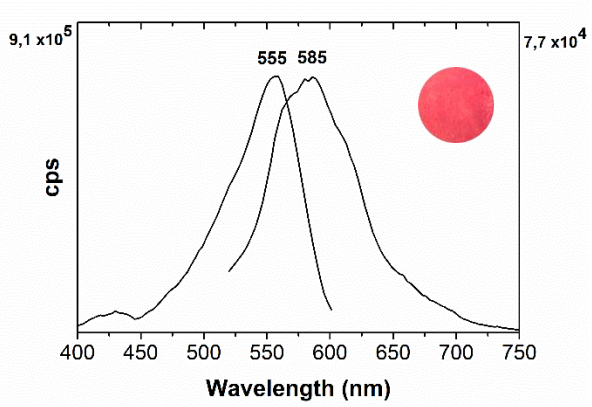
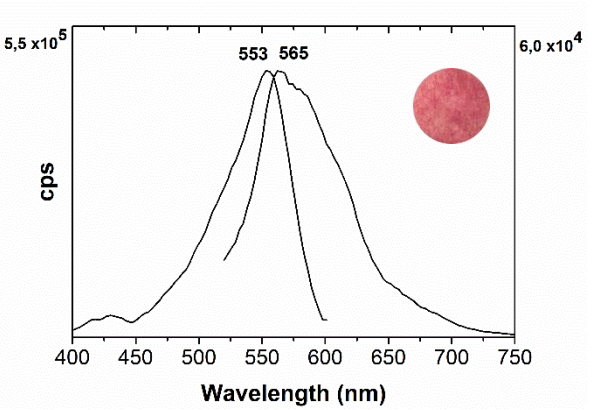
Detail from the Teology Treatise, 14th century
fol. 43r © Fondo Ka'ti

Appendix 1. Microspectrofluorimetry for the preservation of red lake pigments

A1.1. Historically accurate reconstructions database

Recipes translations and transcriptions

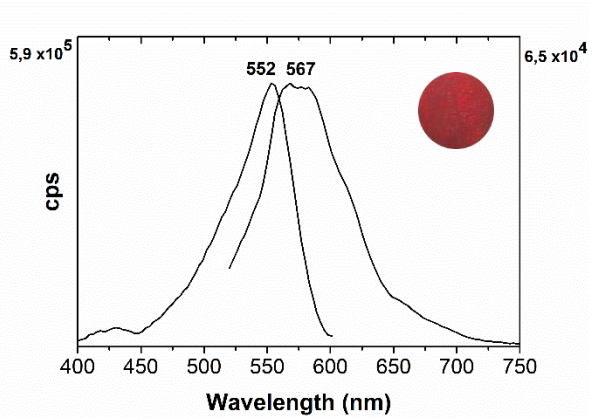
Table A1.1. Brazilwood reconstructions from medieval treatises. English translation of the recipe followed by representative excitation and emission spectra. Reconstructions prepared within the scope of the MsC thesis of Tatiana Vitorino (Chapter 2, references [5, 18, 32]).

‘*The book of all color paints*’, O livro de como se fazem as cores das tintas todas (15th c.)	
Chapter 8. In order to make rose	
In order to make rose, take one ounce of fine brazil-wood, and scrape it very fine, and set it aside. And then take a quarter ounce of alum and take two pennyweights of white lead and grind it with the alum in a mortar and set it aside. And then take the brazil-wood and place it in a cup [made] of *malega* and put in the other powders with the brazil-wood and pour urine over them until they are covered. And let them stand thus three whole days, always stirring them with a stick 5 or 6 times each day. And then pass it and strain it through a linen cloth above a trough made of gypsum or chalk-stone. And let it soak in the trough, and when it is dry, scrape it very well with a spatula, and keep it carefully from the air. And when you want to work with it grind it with gum water.	
‘*The book of all color paints*’, O livro de como se fazem as cores das tintas todas (15th c.)	
Chapter 9. In order to make another rose color	
In order to make another rose color, take brazil wood, as much as you need, and scrape it very fine and place it in a small new pot. And place in the pot lye of vine branches, so that the brazil wood is covered with it. Put it on the fire and heat it enough for the lye to take up the substance of the brazil wood. And take two part of alum and at least a half a part of chalk and grind each one well by itself. And then mix it and grind it together, and make, as you already know, rose out of alum.	

'*The book of all color paints*', O livro de como se fazem as cores das tintas todas (15th c.)

Chapter 27. If you wish to make good rose-color

If you wish to make good rose-color, take brazil-wood, as much as you want, and scrape it well over a conch or retort, then add alum to it. And once you have done this, take the urine of a chaste man and add pour it over the brazil-wood and the alum until they are well covered, and let them stand thus for three days. And then take a piece of chalk and throw some of its powder over this brazil-wood until it seems that there is as much of one as of the other. And then let this concoction stand for a day or two. And then take this rose-color and grind it with gummed egg-white and write with it. If you wish to make indigo, put blue in it, and if perhaps you wish to make a black color, put black in it. And if perhaps you



wish to turn white color black, add black to it and white and take brazil-wood and place it in a white cloth and strain it over chalk. Know that the principal colors are ten: blue, orpiment, and vermilion, green, Sufi carmine, sunflower, saffron, red lead, white lead, brazil-wood. When you want to thin egg-white add the sap of a fig tree to it, and thin it very well, clear as water, for your work.

'*The book of all color paints*', O livro de como se fazem as cores das tintas todas (15th c.)

Chapter 44. If you wish to make good rose-color

If you wish to make good rose-color, take brazil-wood and grind it in a mortar, until it is well ground. Sift it and take a little virgin lime and place it in a glazed earthenware bowl with water until the water becomes clear, and with this water grind the brazil-wood, and put in it a little alum, temper it with gum, and write with it.

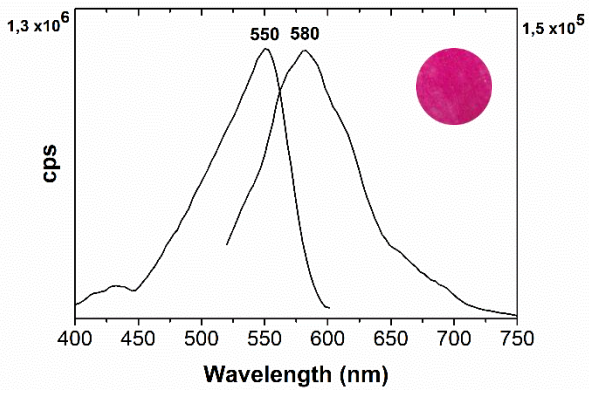


Table A1.2. Cochineal reconstructions from 19th c. Winsor and Newton database. Transcription of the recipe followed by representative excitation and emission spectra. Reconstructions prepared within the scope of the PhD thesis of Tatiana Vitorino. The transcriptions of the recipes can be found in this thesis (Chapter 2, reference [22]).

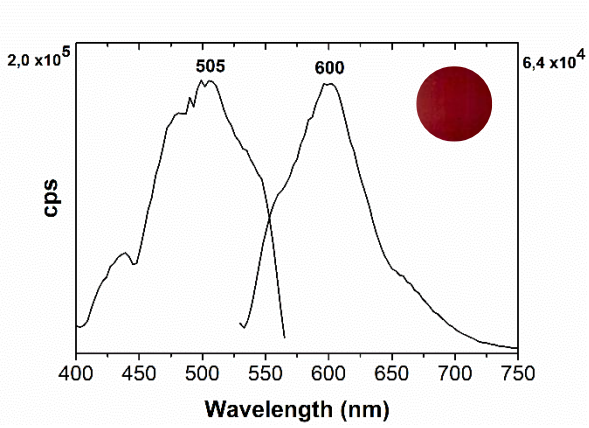
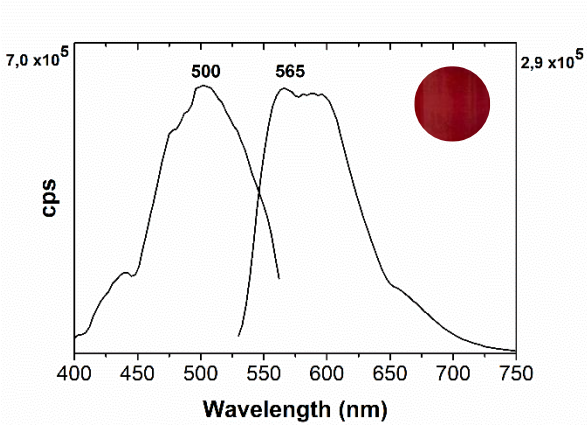
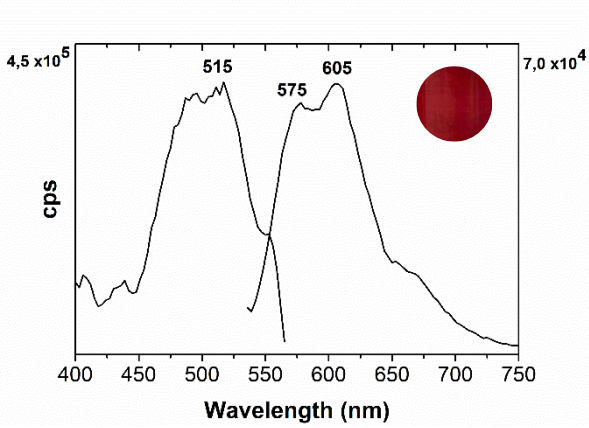
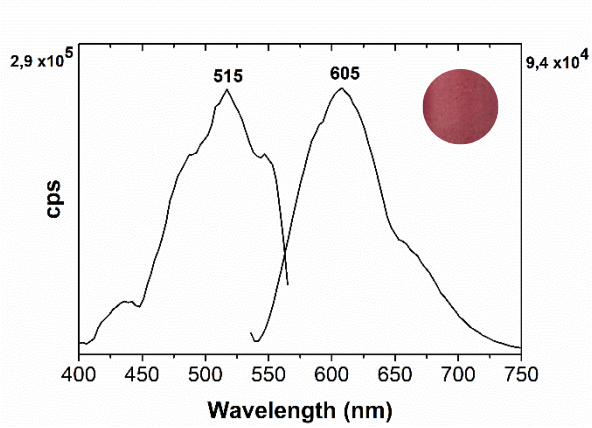
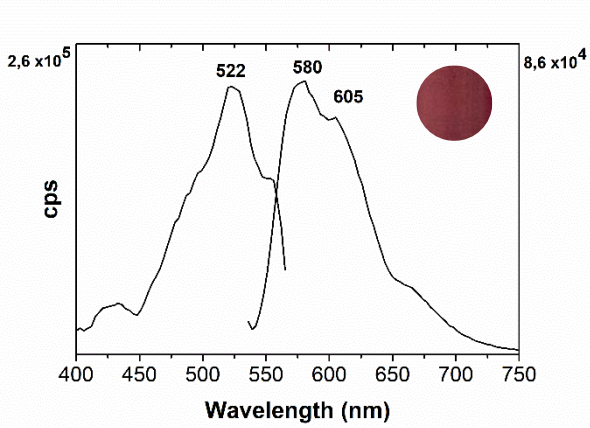
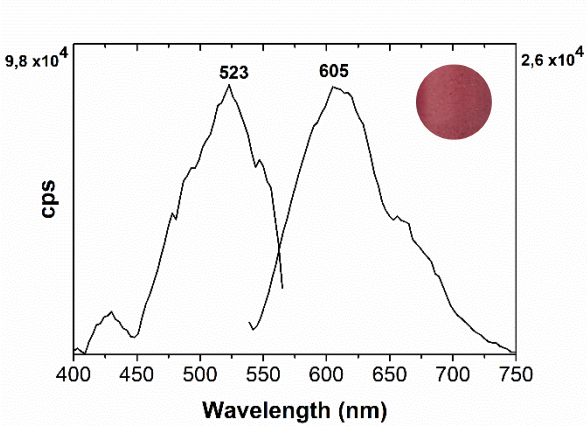
Finest Orient Carmine	*Half Orient Carmine*
	
Ruby Carmine	*Ruby Crimson*
	
Crimson 1	*Crimson 2*
	

Table A1.3. Kermes reconstructions from a medieval treatise. Transcription of the recipe followed by excitation and emission spectra: *left*, applied with glair; *right*, applied with gum-arabic. Reconstructions prepared within the scope of a MsC class, (Chapter 2, references [30, 31]).

Ms Jean le Begue (1431)

Recipe 11. To make fine lake

Take the ashes of oak, and make a ley, and boil in it clippings of fine scarlet of *rubea de grana* until the color is extracted from the clippings. And then strain the ley with the color through a linen cloth. Afterwards take some ley, similar to what you first took, and heat it; and put into it some powdered *roche alum*, and let it stand until the alum is dissolved. Then strain it through the strainer with the other liquor or ley in which the clippings were put, and immediately the ley will be coagulated, and make a lump or mass, which you must stir well. Remove it afterwards from the vase and lay it on a new hollow brick, which will absorb the ley, and the lake will be left to dry. You must afterwards take it off the brick and keep it for use.

Roossen-Runge adaptation (1967):

300mg kermes insects are first pulverized as finely as possible in a mortar. Then 5 mL of saturated lye from oak ashes is added, and both again triturated finely to a smooth liquid. The liquid is strained through a fine linen cloth so that the shell particles are removed. Then an additional 5mL saturated oak lye is heated, and 150mL alum powder added to this liquid. After the alum has dissolved in the lye, the latter is again strained through a fine linen cloth into the kermes liquid obtained in the first operation. The precipitate separates immediately, producing a colored mass from the colorless clear liquid. The pulpy colored mass is set on a dry shard of a fired clay por, which absorbs the liquid, leaving a dark red substance that can easily be scraped off.

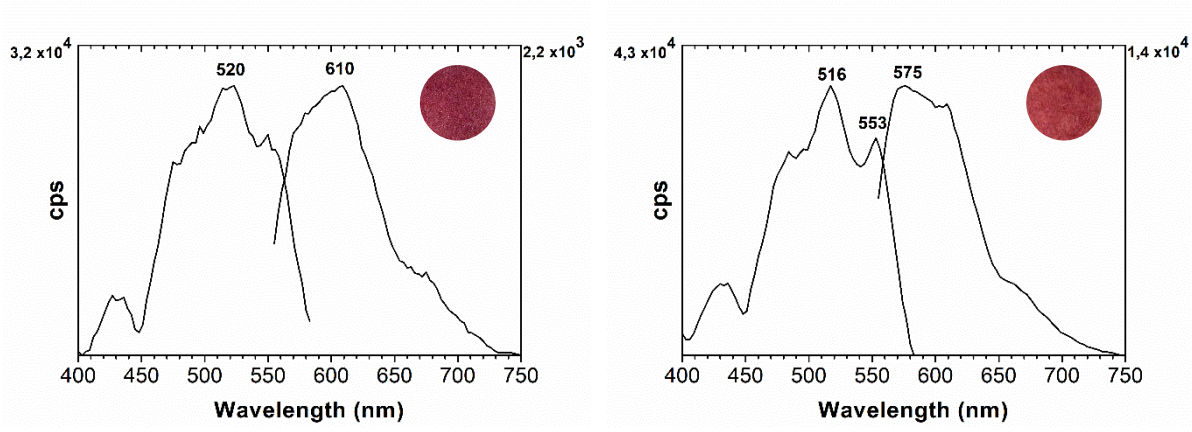
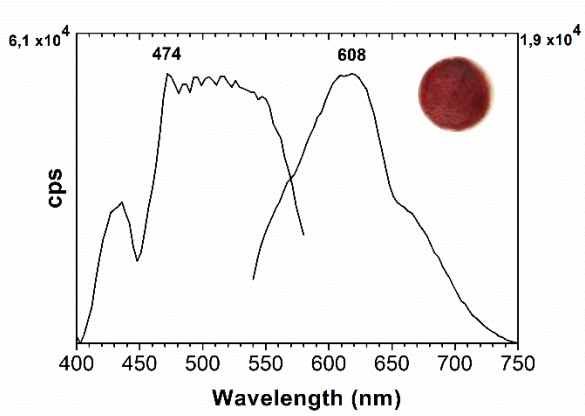


Table A1.4. Lac dye reconstructions from medieval treatises. Transcription of the recipe followed by excitation and emission spectra. Reconstructions prepared within the scope of the PhD thesis of Rita Castro (Chapter 2, references [9, 20, 31-38]).

Ibn Bādīs (c. 1025)

Chapter 6. On the mixture of dyes, colors, and their preparation

Another color is red ruby from the *lukk*. How it is made. Description of how to dissolve the *lukk*. Ten ounces of *lukk* are broken up after it has been freed of its twigs. Then two dirhams of *ushnān* and two dirhams of *bauraq* are pounded very finely. Enough water is poured on to cover them. It is brought to the fire with the *lukk* until all the redness of the *lukk* is brought out. It is removed from the fire. It is filtered, returned to the fire, and boiled until half of the *lukk* solution remains. It is then removed. Write with it. If it is desired that it remains dissolved, a piece of hard white sugar is added to it. If it is desired dry, it is placed in the shade protected from dust. When it is dry, it is removed and used for that which is desired.

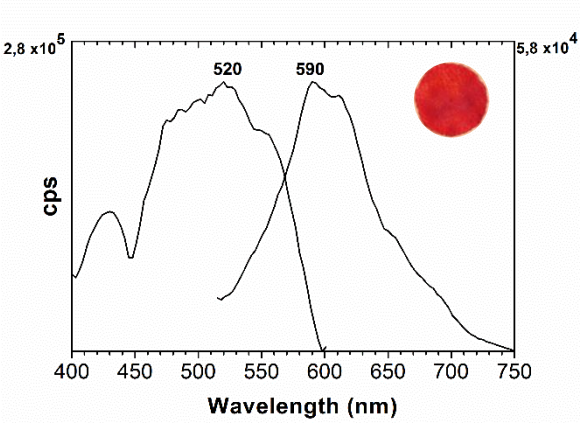


The *lukk* is broken into pieces and powdered like the crumbled chick pea. It is washed with water and put in a thick filter. The water is boiled vigorously. While it is in the filter, hot water is poured on it so that its color, red, will flow from the filter. The filtrate is boiled until it is decreased by two-thirds. Then dissolved gum is melted in it. Write with it. It comes out well.

Ms Mappae Clavicula (12th c.)

Recipe 253. Lac, how it is worked for painting on wood or on a wall

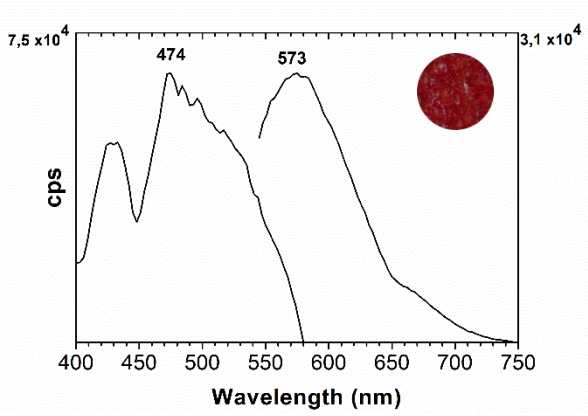
First grind lac and from it pick out the knobbly bits and impurities; then put it in a mill and grind it fine; then take the urine of a man or woman and first put it in a cauldron and let it boil until it is all reduced to a third. Continue always to take off the froth. Afterwards put in the lac and let it boil; then take very clean alum and grind it and mix it in the above-mentioned lac. Then take a small cloth and keep on dipping it until a good color appears [on it]. Then put the liquid into little pots and work [with it]. Throw out the stone which forms in the liquid, because it is of no value. Into 5 pounds of lac put 5 oz. of alum and 10 pints of urine.



'*The book of all color paints*', O livro de como se fazem as cores das tintas todas (15th c.)

Chapter 13. In order to make fine carmine, '*para fazeres nobre karmen'*

In order to make fine carmine, take a large new pot that holds four *açumbres* of water, and fill it with human urine. And mix it for days and make it very clear all the while so that it gives off foam. And once it is very clear and skimmed, take a large bowl and place rye-straw over it, and above the straw a linen cloth. And on the cloth place ashes of vine branches, two parts, and a third part quicklime, and place a pot underneath. And throw on the lye the strained urine that you strained through *fried meat* and continue straining it until the pot is full of this strained lye, in such a way that there are four *açumbres* of it there. And place it on the fire until only two fingers of it are left, and on the fire put another pot full of clear urine

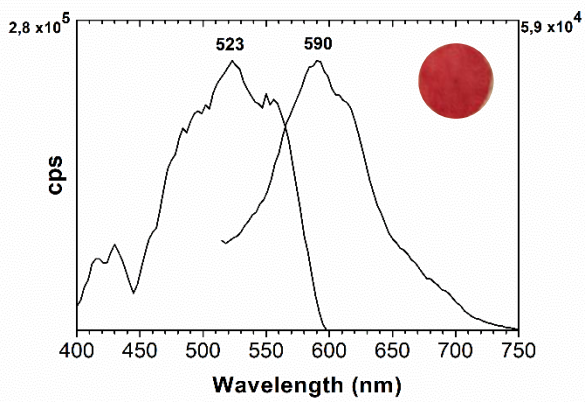


with the strained lye and heat both. And into the pot of clarified urine with the strained lye toss one pound of lac, and heat it gently, all the while stirring it with a slightly forked stick. And when the lac is melted, strain it with a linen bag, and place a basin underneath; whatever remains in the bag, place it in the pot of strained lye, which you have kept on the fire with gentle heat, until it is melted, stirring with a piece of wood. And then strain it separately with the bag containing the powder. Thus you can make carmine of two kinds, though first you must clarify the urine.

Ms Jean le Begue (1431)

Recipe 36. To make lake

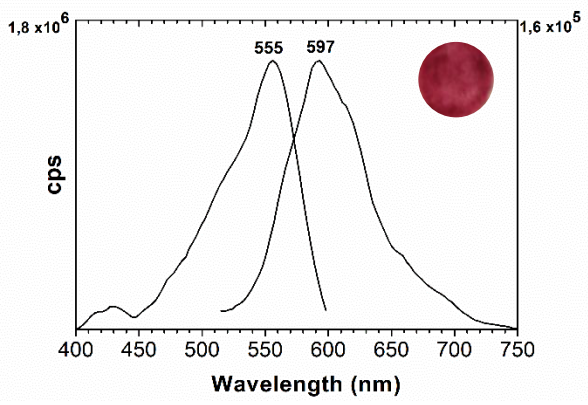
Take urine, and keep it for a long while, and afterwards make it boil until half of it is evaporated upon a slow and clear fire, skimming it continually, until it is perfectly purified. Then strain it through a linen cloth and put 4 lbs. of it into a glazed jar of the said urine, and 1 lb. of raw lac well ground, and add to it a sufficient quantity of *alumine zuccarino*, and put it by and keep it for use.



Ms Jean le Begue (1431)

Recipe 309. To make a very good lake

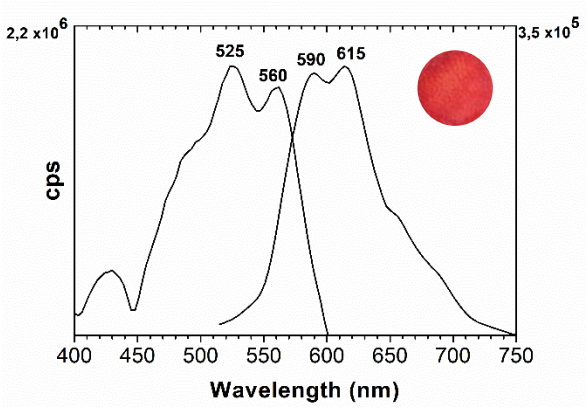
Take an ounce of lake, and rasp finely a little Brazil wood, put it into a clear vessel, then add to the Brazil wood some clean and clear beaten white of egg and a little alum water. Grind the lake with that water and dry it in the sun, and when you wish to use it, distemper it with this water, especially on parchment, and the more you grind it up with this Brazilwood water, the better it will be.



Ms Bologna (15th c.)

Recipe 129. To make good lake

Take of urine as much as you like and put it into a vase for the space of a week; then pour it into a pipkin and make it boil until no more scum arises. Then make it into a ley with strong ashes. Next take raw gum lac and pound it as small as panic, put it into a new glazed pipkin, and add to it some of the ley of urine, which must be quite clear, and mix it well with a stick; let the urine or ley be warm when it is poured upon the gum, and when it is well mixed, pour off gently the ley so colored, and put it into a glazed jar. Then take *roche alum* in fine powder and mix it with water; then put some of this alum water into the shell containing the ley colored with the lac, and when

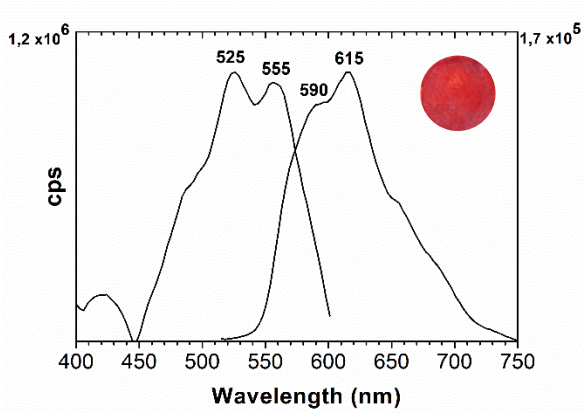


you see that it begins to froth, do not put any more. Then put that which has coagulated into a piece of linen like a strainer, hang it up high, and let the water run off; then take the drainings and put them back into the pipkin where the gum was still left, and mix it up well. Then pour it out, and repeat this another time, thus making 3 sorts of lake; the first best; the second not so good; and the third worst. And know that the ley must be poured very strong, made with urine, and baked ashes, and it must be poured very hot upon the powdered gum, putting the gum on a strainer or filter of linen; then pour the hot ley several times upon it; afterwards add the alum, and dry it; and also dry by itself what remains in the strainer, and it is done.

Ms Bologna (15th c.)

Recipe 130. To make lake as before in another manner

Take of gum lac 5 lbs., reduce it to powder and sift it through a close sieve; then take filtered urine, which has stood for 20 days, and place a small kettle on the fire, into which put the urine, and when you see the scum which floats upon the urine, remove it with a perforated ladle, and when the urine is well skimmed and warm, add 3 oz. of *roche alum* in powder, and make it boil again, and then again while it is still boiling take off the scum with the ladle, and when it is well skimmed and clear, take gum lac, sifted, and put it into the urine and alum, continually mixing it over a slow fire for the space of 3 misereres. Then take it off the fire and put it into a clean wooden powder, either rasped or pounded in a bronze mortar,

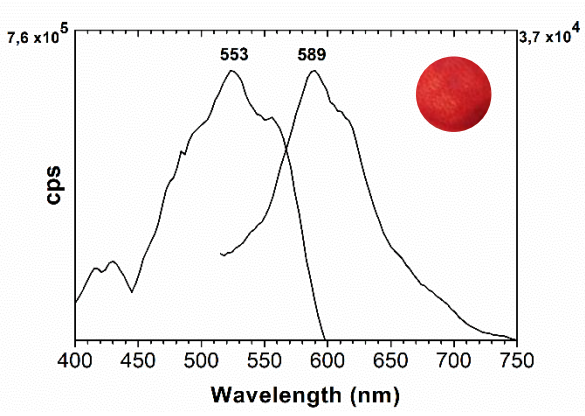


and put it over the fire in a small glazed jar with a little water, and make the said *verzino* boil; afterwards strain it into a vase through a thin and close woolen cloth, and let it cool for one natural day; then take the urine with the alum which is in the before mentioned wooden bowl, and put into it this water which has been boiled with the *verzino* and then strain and mix it well together. Afterwards take 2 lbs. of *roche alum*, and put it into two *metadelle* of clear water, boil it, and afterwards put the alum water into the urine, and mix it well and let it settle for a day; strain it through a strainer and let it settle for another day. Then let it dry, and when nearly dry, cut it into pieces as you please, and let it dry hard. And observe, that you may make lake in this way from various stones and of various kinds, namely, from that from which the crimson color is made, from dragon's blood, from grana, from *vermiculis*, from *minio*, from *verzino*, and from the flowers of herbs.

Ms Bologna (15th c.)

Recipe 131. To make lake by another process

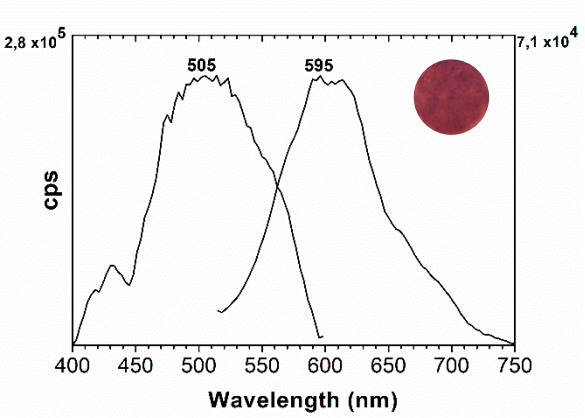
Take urine, pour it into a new glazed jar, and put it over the fire, make it boil well, and while it is boiling take off the scum which arises with a stick, and let it boil until one half is consumed. Afterwards, put the gum lac into the urine, and let it boil with a small quantity of gum Arabic, and a little alum *zucharino*, or *roche alum*. When it has boiled for the space of one hour, strain the liquor through a thin linen cloth, and let it settle in a glazed jar, and the lake will sink, that is, will go to the bottom. Then pour off the urine which remains upon the lake, taking care not to pour off the lake also, and let the lake dry by itself, and not by the fire, nor in the sun, and it will be good and perfect lake.



Ms Bologna (15th c.)

Recipe B 137. To make lake

Take one ounce of crude lake or grana, put it into a small pipkin, and pour on it sufficient urine or ley to cover the lake, and make it boil on a moderate fire for half an hour without smoke. When it boils keep mixing it, and when it has boiled take ½ oz. of *roche alum*, and ½ oz. of sal gem, and grind them well with ley, and put them into the jar before it has done boiling; then take the vessel from the fire immediately and let it cool. Next take a wash-hand basin and a *petito* of stale urine, or a strong ley, and throw the whole into the basin, and mix it together, and stir it very well with a stick, and put it for 15 days in a place free from dust, stirring it every evening and morning; at the end of a fortnight take a small linen bag and

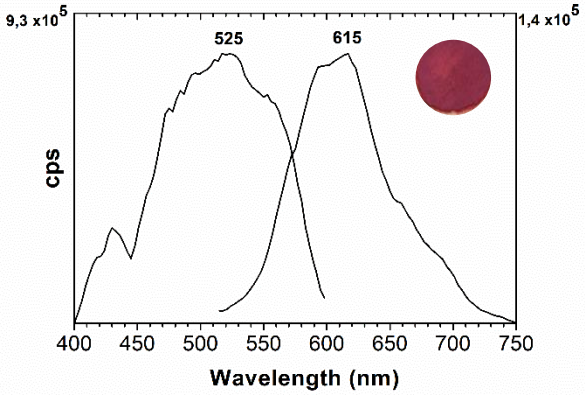


strain it and put what you have remaining on a new and clean tile, and dry it directly in the shade, and you will have fine lake. Put it back into a box and cut it into pieces, &c.

Ms Bologna (15th c.)

Recipe B 140. To make lake in another manner

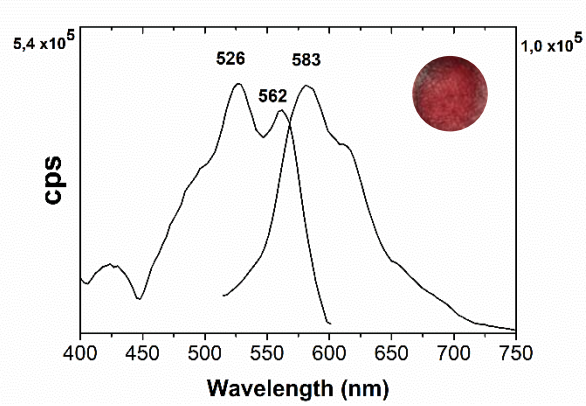
Take 1 lb. of gum [lac], and put it into very strong boiling ley, and let it dissolve; then take three glasses of tepid water in which 2 oz. of *roche alum* have been dissolved, but first put the water into a large shell, throw on it the boiled ley, and let it remain so for 2 days; then take a glass and take also that gum and water and ley, and strain it in a small woolen bag; let it run through and the lake will remain at the bottom.



Ms Strasbourg (15th c.)

Bright Paris Red

If you want to make beautiful and fine Paris red. First, you should make a lye from willow ashes. Take a species which is called '*lagga*' with which one dyes the leather. One should grind it into fine powder. One should heat the lye and pour the powder of the '*lagga*' into the hot lye and stir it all together and let it stand over night for pickling/(soaking). In the morning, one should put the color on the fire and one should stir it without interruption. One should boil it half the time as one cooks a fish. Then one should put a *settin of ice alum* in the color and one should stir it until it dissolves. After that take the color from the fire and let it cool down. Filter the color through a clean

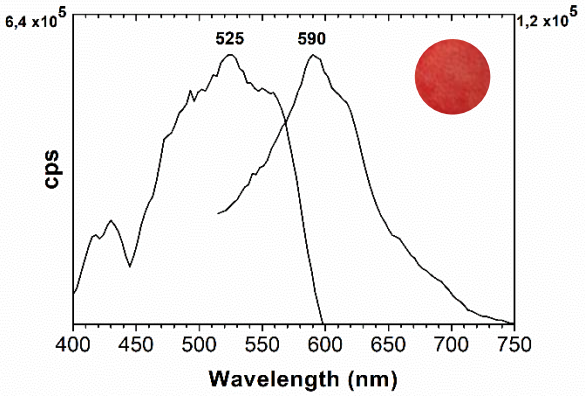


double-layer cloth. Wring the color through it into a glazed vessel. Take the alum which should be finely ground and stir slowly the powder into the color, stir it with a little spoon until the alum is quite well dissolved in the color. Here note a sign; if the color becomes thick as a warm wine and already beautiful red, then one should no longer add alum to it. (But) if the color is thin fluid like water then one should add more alum into it and stir together until the color becomes beautiful (and) thick. After that pour the color all together into a bag whose form is identical to that of the bag (used) for the rose color. Hang up the bag in the same way for draining over night until nothing drains out anymore. What drains from the bag is (light red) as light red wine. One should drain it away. But what remains in the bag is a beautiful and fine red. One should turn over the bag and put the color on the stone and scrape the color with a knife from the bag. Then put the color in the wind and let it become hard. Then keep it clean until one needs it. (This color is called red of Paris, this is the most expensive one finds).

Ms Montpellier, *Liber diversarium arcium* (15th c.)

Recipe 1.9. On the preparation and tempering of *lacca*

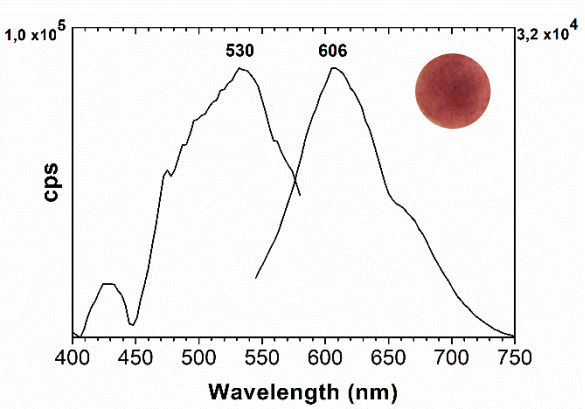
We shall now proceed to the preparation of *lacca*. (…) Grind that well, then take two vessels and put in plenty of urine and boil it, and skim the urine well with a feather, and boil it, which urine shall be of a twelve year old boy drinking good wine; when, then, it shall be well skimmed, take a third part of the aforesaid urine, and then take *zucharino* alum and mix it in urine and pour it into the other [vessel] on the fire, and cook; we recommend cooking for a long time on the fire; when, then, it shall become good and red, take it from the fire and strain through a long linen bag, and again add cold urine with some divided alum [i.e. alum pieces], note: with the discharge is added [either one or ten ounces (?)] of gum Arabic, which thickens it.



Ms Paduan (16th – 17th c.)

Recipe 90. To extract the color of the gum lac, so that it will serve for bright colors

Pulverize coarsely the gum, and put it into a ley of rosewood or vinewood, which will extract its color; you must then separate the water from the gum, and evaporate it carefully until it begins to thicken. You must then take it from the fire, and stir it with a silver spoon, and let it settle till the next morning, and when it is sufficiently thick, you must place it on a slab of marble, dry it and use it for giving a luster to the gum and to all colors. Observe that this color mixed with white lead makes a beautiful flesh color. The remainder of the gum lac must be washed in the ley until it has entirely lost its red color; it will then do for melting with the other colors, as cinnabar, orpiment, and others, because the gum being passed through

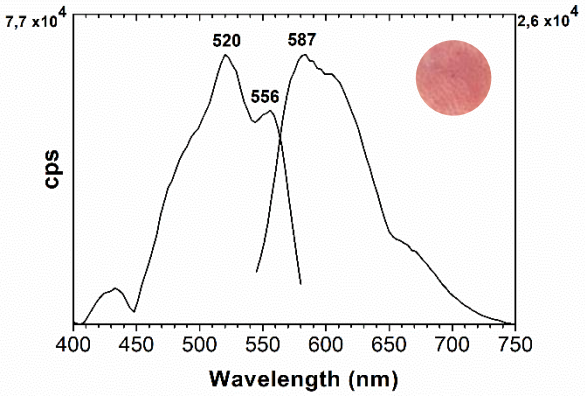


the ley, is clarified [or bleached], and all the impurities of the inside will pass off with the color.

Ms Paduan (16th – 17th c.)

Recipe 113. To make very fine lake

Take ½ oz. of lac, half a drachm of crystals of tartar pulverized, and a *scudella* of hot water. First dissolve the tartar, then take the lac which has been ground, put it on a clean linen rag, and tie it into the form of a ball, and then cut off the superfluous part of the rag, and put the ball into the above-mentioned hot water, placing the *scudella* over some hot cinders, and leaving it there until the water becomes well colored. When this is the case, take the ball from the water, and evaporate the water gently over the hot cinders until the color is condensed at the bottom; it will then be done.



Lac dye characterization

Lac dye shows just how a signal from a chromophore depends on its environment. Different recipes lead to different signals. To better understand these differences, one must look at the simple components. Lac dye is a resinous substance secreted by insects of the *Kerria* spp [Chapter 1, ref. 9]. The resinous material is known as sticklac in its raw form, but as shellac in a refined state. This latter, besides laccaic acids A, B, C, D and E (the first two in higher amounts), also contains erythrolaccin, a yellow orange chromophore which characterizes the resin, **Figure A1.1**.

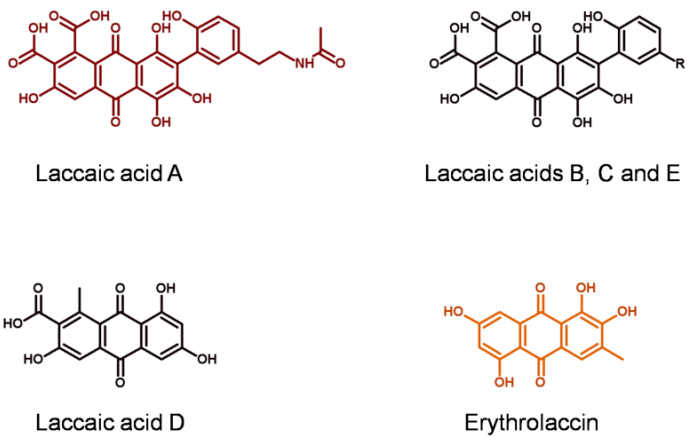


Figure A1.1. Chemical structures of laccaic acid A, laccaic acid B, C and E (B, R=CH_2CH_2OH; C, R=CH_2CHNH_2COOH; E, R=$CH_2CH_2NH_2$), laccaic acid D and erythrolaccin.

Castro separated the lac dye recipes in three main types based on her research using complementary techniques, such as infrared, SERS and fluorimetry: **type A** – alum-lac complexes (e.g. Mappae Clavicula, Bolognese 129, Montpellier); **type B** –free lac reproductions, with resin (e.g. Ibn Bādīs, Book of all color paints); **type C** – alum-lac complexes with brazilwood (e.g. Jean le Begue 309, Bolognese 130). Therefore, understanding how a signal is changed based on these types, is very important to better understand the paint color.

When comparing the excitation and emission spectra from the shellac and from lac dye complexed with Al^{3+} we can see clear differences, **Figure A1.2**. For the first, the excitation spectrum is broad and unresolved, with a maximum at 472 nm, and an emission at 586 nm. According to Castro [Chapter 1, ref. 9], these data match that of literature for erythrolaccin, characterized by a maximum at 464 nm. Regarding the Al^{3+}-lac dye complex solution applied to filter paper, this displays a well-resolved excitation spectrum, with maxima at 530 and 565 nm, and emission maximum at 585 nm and a shoulder at 612 nm. This indicates a small Stokes shift, indicating that the same species was absorbing and emitting in the excited state. Interestingly, according to Castro [Chapter 1, ref. 9], the relative intensities of the excitation spectra may change with different amounts of alum: the higher the amount, the more intense the spectra.

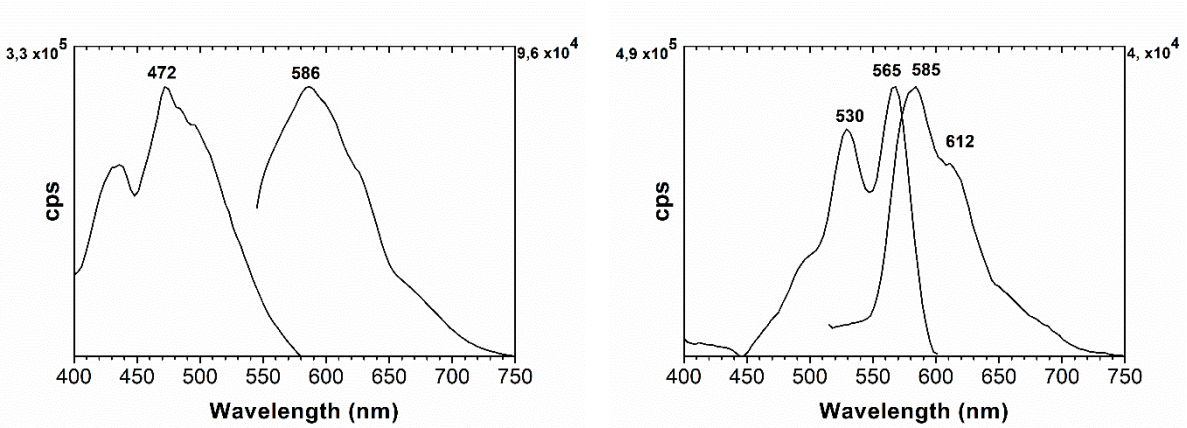


Figure A1.2. Normalized emission and excitation spectra of shellac (*left*) and Al^{3+}-lac complex (*right*)[80].

Infrared spectroscopy can also be very helpful in identifying the resin present. The fingerprint of shellac is clear, as can be seen in **Figure A1.3**. Its main characteristic bands are observed at 2931 and 2858 cm^{-1} from the CH stretching absorption bands; at 1716 cm^{-1} from the C=O stretching vibrations; at 1637 cm^{-1} from the CO stretching vibrations; at 1560 cm^{-1} from the CN stretching and NH bending; at 1466 cm^{-1} from the C=C stretching aromatic rings; at 1387 cm^{-1} from the CH bending rings; at 1255 cm^{-1} and 1045 cm^{-1} from the C-O stretching carboxylic acids [Chapter 1, ref. 9].

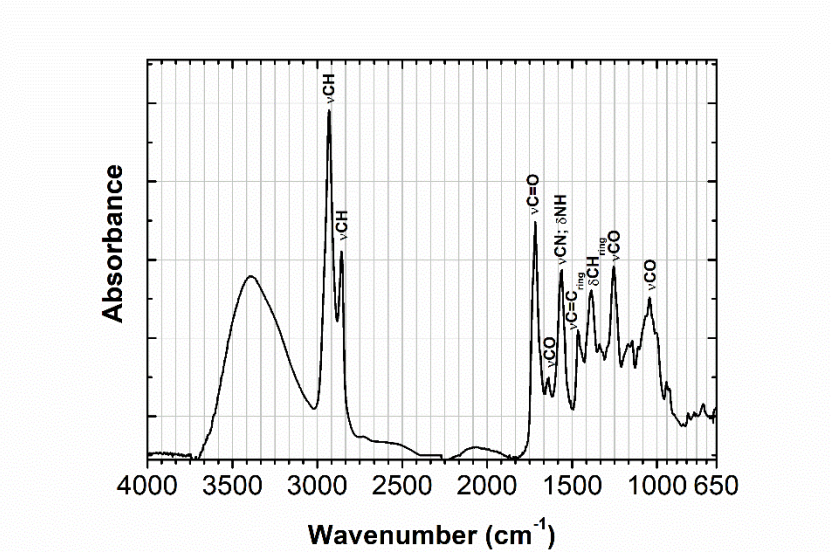


Figure A1.3. Infrared spectrum of a shellac standard applied on filter paper at pH=9 in ethanol[81].

[80] The data was acquired by Rita Castro.
[81] The standard was prepared, and the data acquired by Catarina Miguel and Rita Castro.

Microspectrofluorimetry database[82]

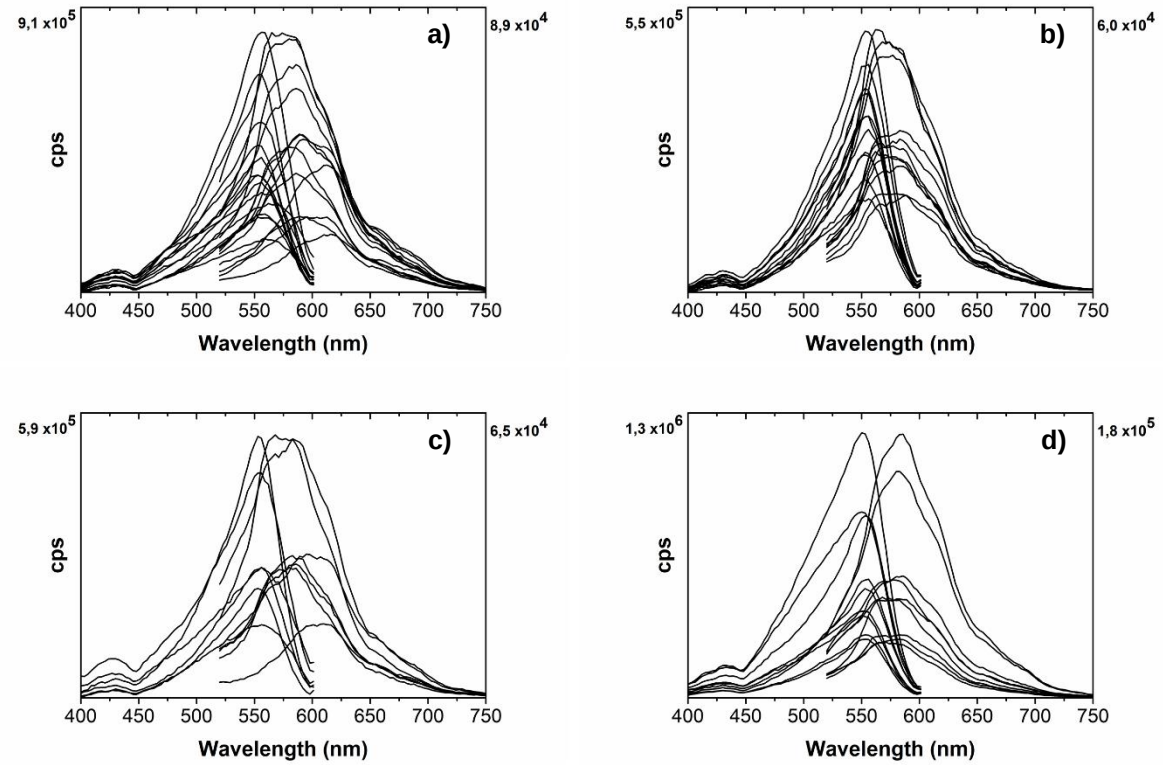


Figure A1.4. Excitation and emission spectra of brazilwood recipes from '*The book of all color paints*': recipes 8 (*a*), 9 (*b*), 27 (*c*) and 44 (*d*).

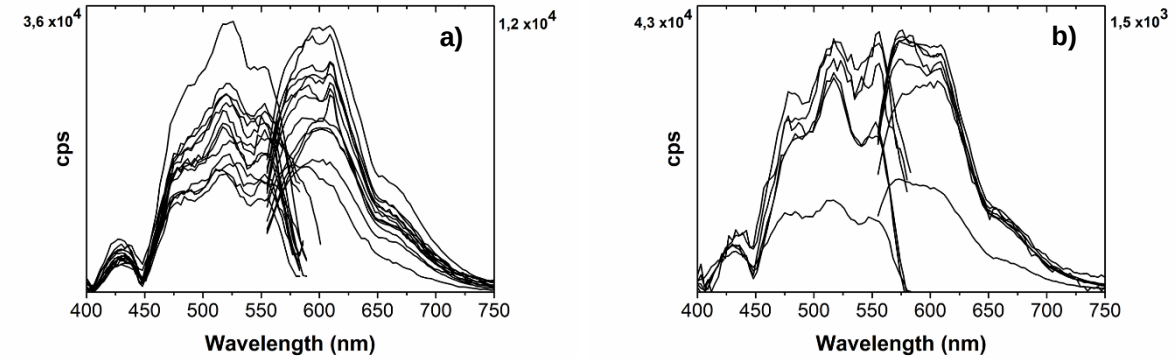


Figure A1.5. Excitation and emission spectra of kermes recipes from the a Roosen-Runge adaptation of a *Jean le Begue´s manuscript* applied with glair (*a*) and applied with gum-arabic (*b*).

[82] The data from the brazilwood and cochineal reconstructions were acquired by Tatiana Vitorino, from the kermes reconstructions by Tatiana Vitorino, Paula Nabais and Artur Neves, and from the lac dye reconstructions by Rita Castro and Paula Nabais.

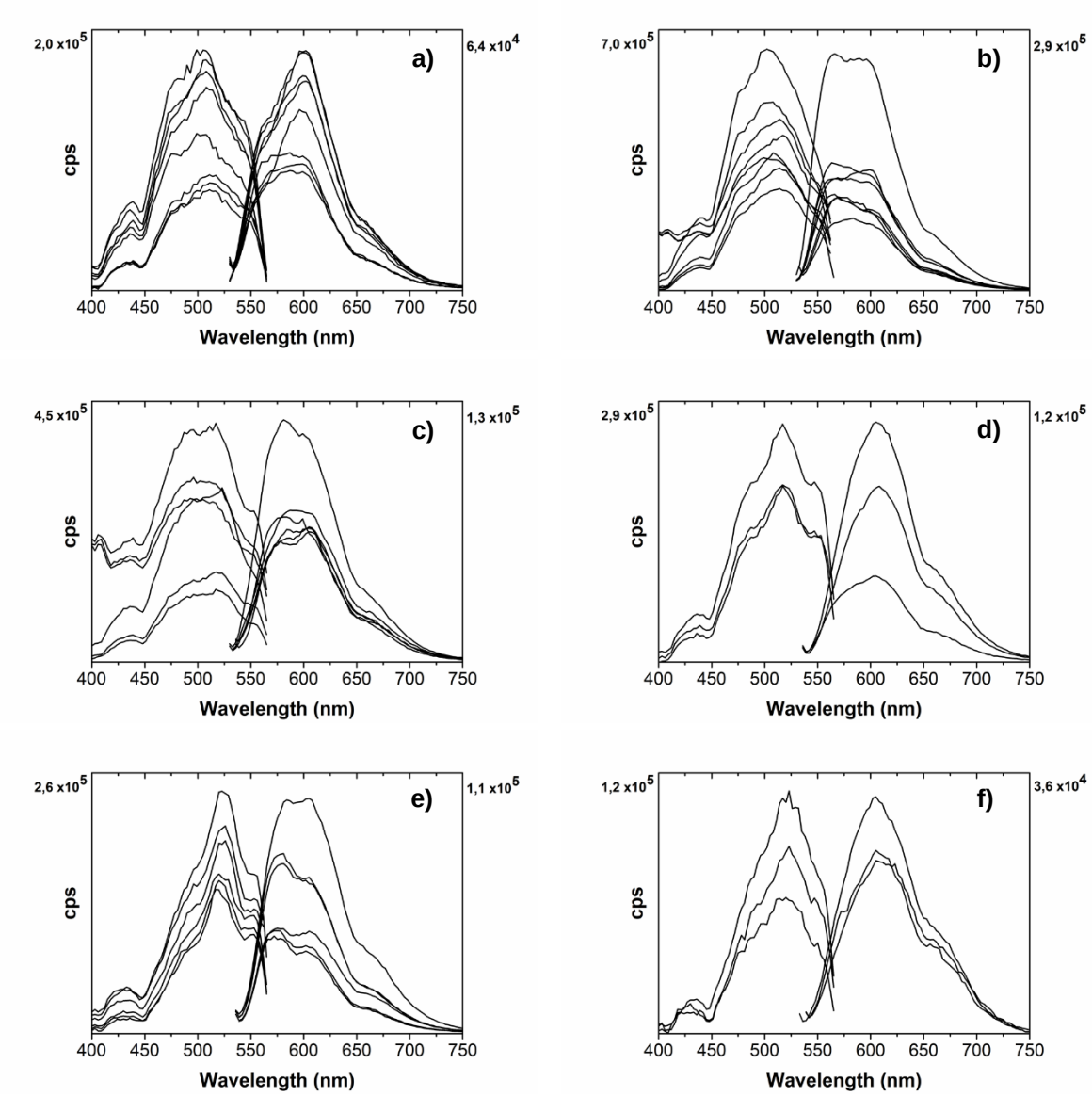


Figure A1.6. Excitation and emission spectra of cochineal recipes from the Winsor & Newton 19th c. archive: *Finest Orient Carmine* (*a*), *Half Orient Carmine* (*b*), *Ruby Carmine* (*c*), *Crimson with gypsum* (lake pigment dispersion with gypsum, *d*), *Crimson 1* (lake pigment dispersion with ammonium carbonate and alum, *e*) and *2* (lake pigment dispersion with sodium carbonate and alum, *f*).

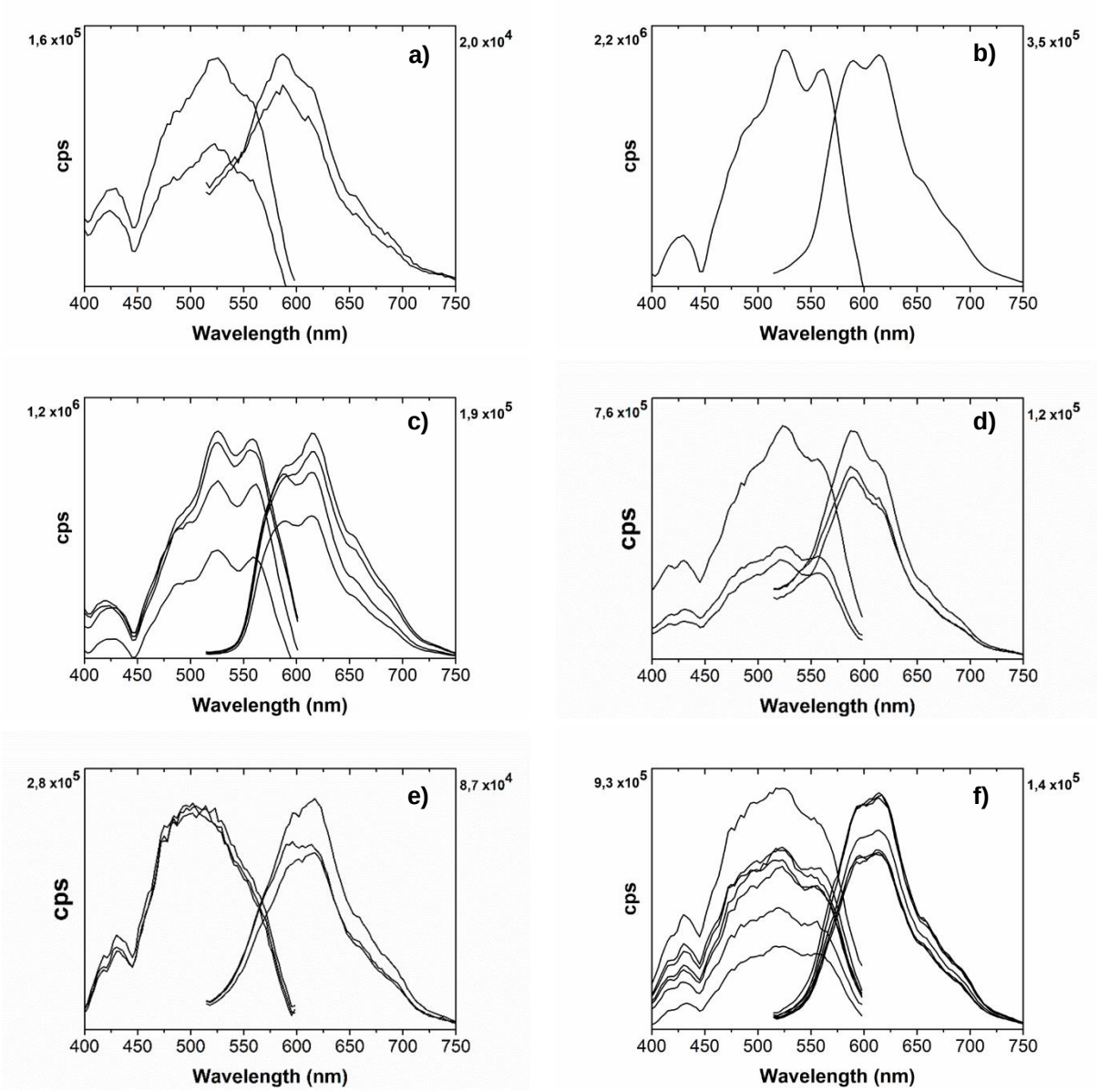


Figure A1.7. Excitation and emission spectra of lac dye recipes: *Ms. Bolognese* recipe 129 (both spectra showing different intensities, *a* and *b*), *Ms. Bolognese* recipes 130 (*c*) and 131(*d*), *Ms. Bolognese* recipes B137 (*e*) and B140 (*f*).

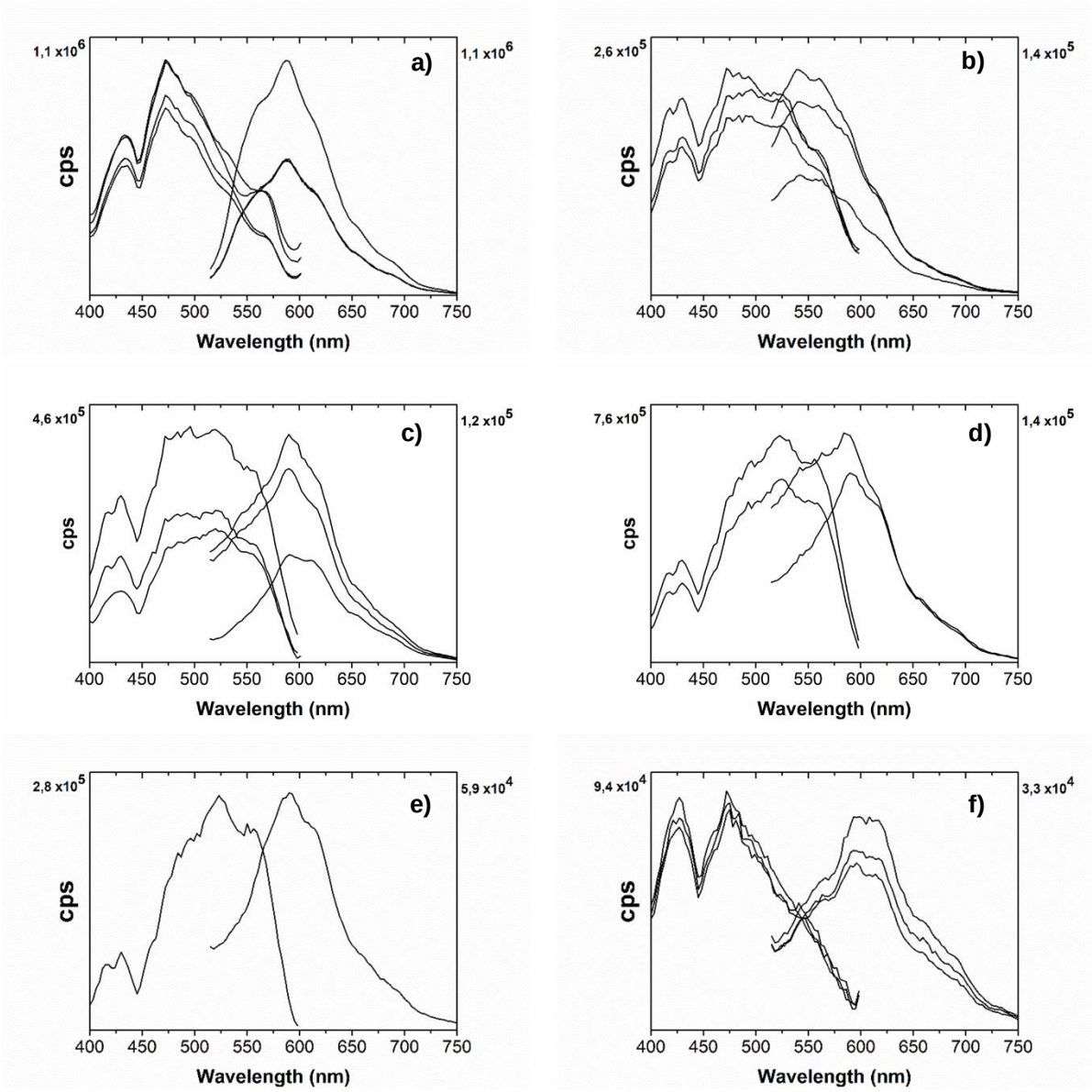


Figure A1.8. Excitation and emission spectra of lac dye recipes: *Ibn Bādīs* chapter 6 (*a*), *'The book of all color paints'* chapter 13 (*b*), *Ms. Mappae Clavicula* recipe 253 (*c*), *Ms. Montpellier*, *Liber Diversarum Arcium*, chapter VIII, recipe 1.9 (*d*), *Ms Jean le Begue* recipe 36 (*e*) and *Ms. Strasbourg* (*f*).

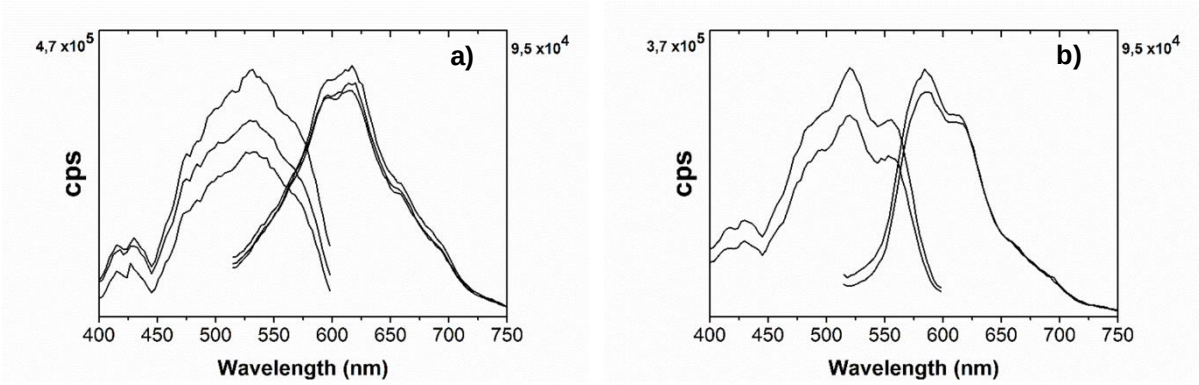


Figure A1.9. Excitation and emission spectra of lac dye recipes: *Ms Paduan* recipes 90 (*a*) and 113 (*b*).

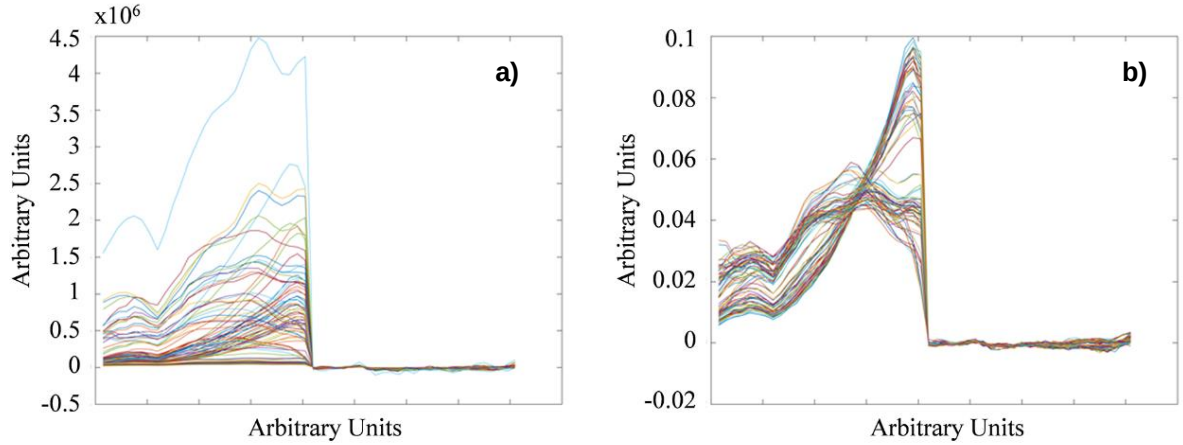


Figure A1.10. Pre-treatment of the excitation spectral set prior to the application of HCA: Haar transform (*a*), and Haar transform + normalization (by area = 1) (*b*).

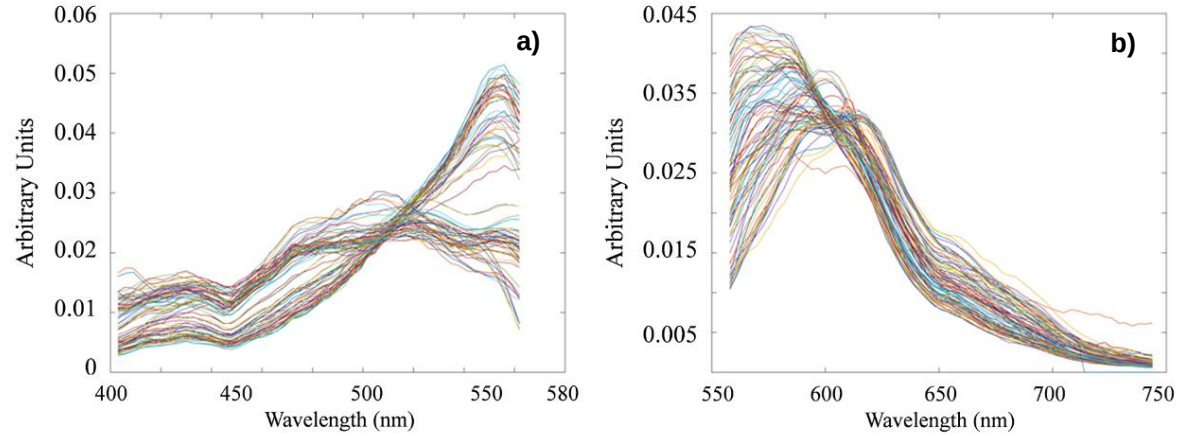


Figure A1.11. Normalization (by area = 1) of the excitation (*a*) and emission (*b*) spectral set prior to the application of the SIMCA modelling.

A1.2. Artworks database

The methodology described in **Chapter 3** was not only tested on medieval manuscripts but also on data acquired from Andean textiles. A collaboration with the Museum of Fine Arts in Boston (MFA-Boston) allowed for the analysis of a collection of samples from 76 reds from Andean textiles, dated from 200 B.C. the 15th c. A.D. and created by cultures of Paracas, Nasca, Wari Huari, Chancay and Lambayeque. Previous study of SEM-EDX screening allowed for the confirmation of the use of aluminum ion as a mordant and microspectrofluorimetry was used, together with HPLC-DAD to enable a conclusive identification of carminic acid. The samples were reanalyzed for this work to obtain better signal to noise ratio. It was possible to obtain far better excitation and emission signals with these fibers than with model painted samples of carminic acid, which is why this database is composed of data from cochineal obtained from textiles.

The HCA method resourcing to Ward's algorithm was used to provide a picture of samples spectral similarity. Considering both the emission and the excitation dataset, the HCA method revealed a successful clustering of the three colorants, as seen in **Figure A1.12Figure A1.12.** Dendrogram generated by HCA applied to excitation spectra, showing three clusters encompassing the three colorants: lac dye (*blue*), cochineal (*green*) and brazilwood (*red*).. It is also possible to observe that the lac dye cluster is divided in two main clusters corresponding to the two forms in which we find the colorant: as a lake pigment, as the lac dye cluster closest to cochineal; and as a free lac, the cluster in the limit of the dendrogram. This emphasizes the possibility for differentiating between recipes of the same colorant. The similarity between lac dye lake and the cochineal clusters is well evidenced by both the excitation and the emission spectra, but because the shape of the spectra differ slightly, it is enough for the model to consider them as different clusters.

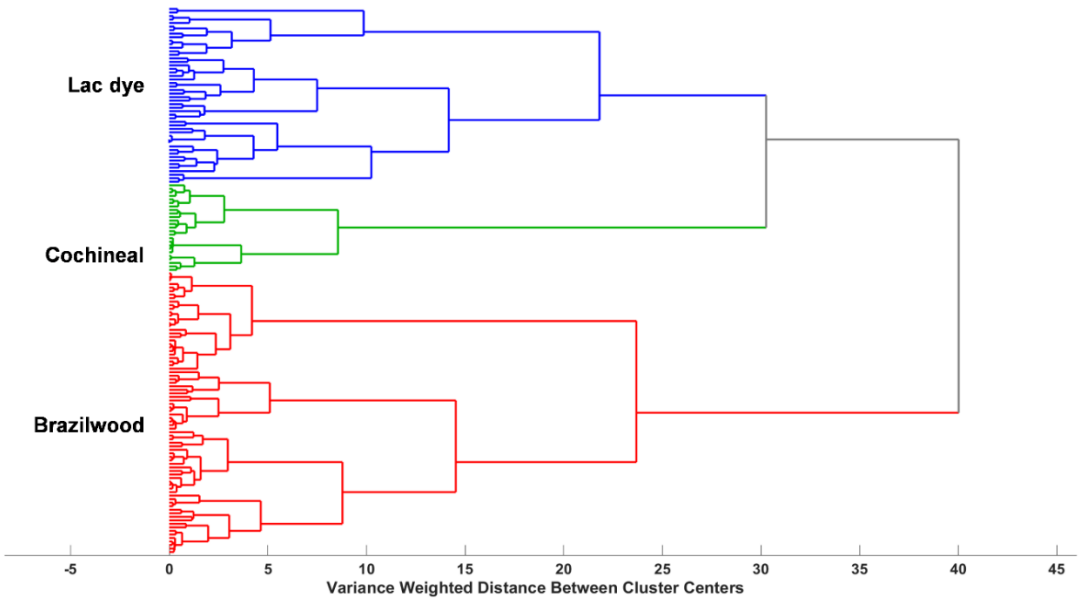


Figure A1.12. Dendrogram generated by HCA applied to excitation spectra, showing three clusters encompassing the three colorants: lac dye (*blue*), cochineal (*green*) and brazilwood (*red*).

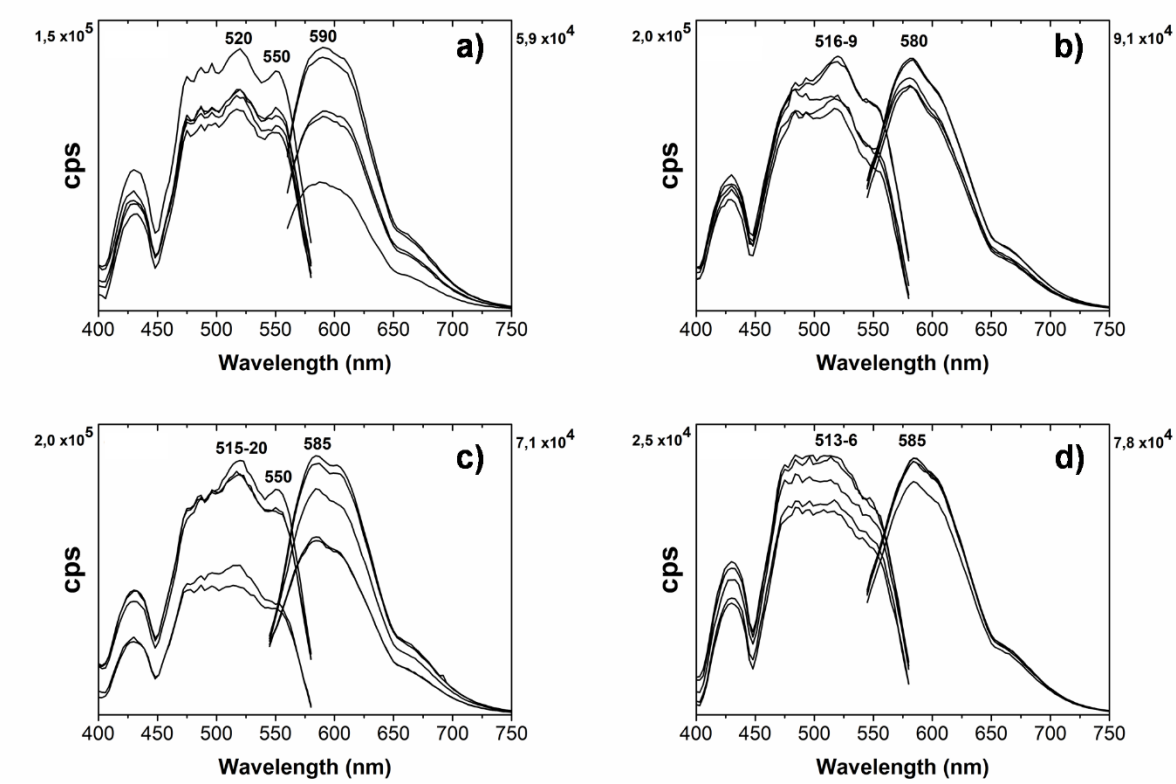


Figure A1.13. Excitation and emission spectra of the selected textiles: 02.680 (s5, *a*) and 47.1087 (s1, *b*), created by the Lambayeque (Late Intermediate Period, 11th – 15thc.); 10.267 (s1 and s2, *c* and *d*).

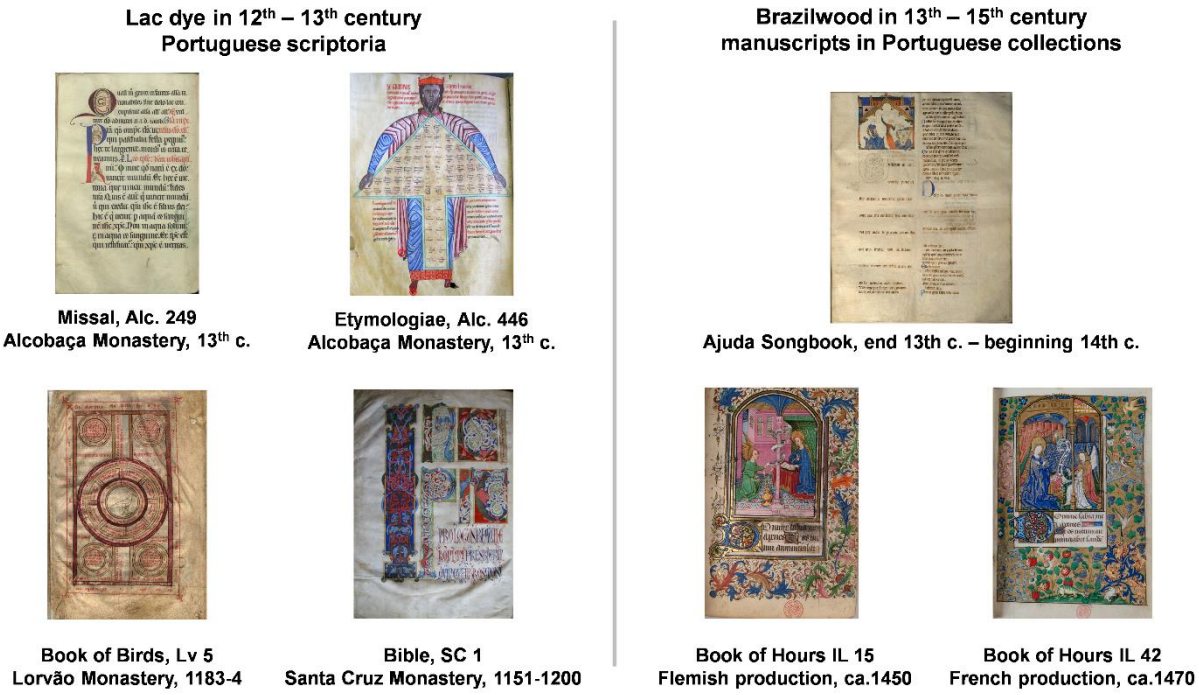
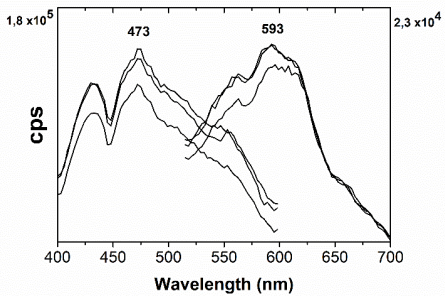
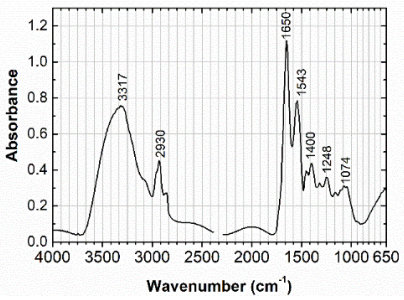
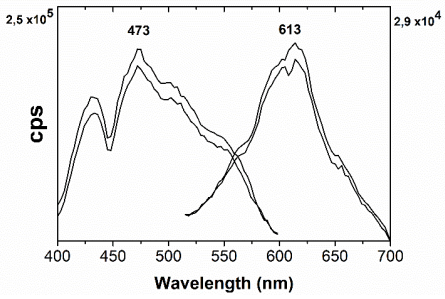
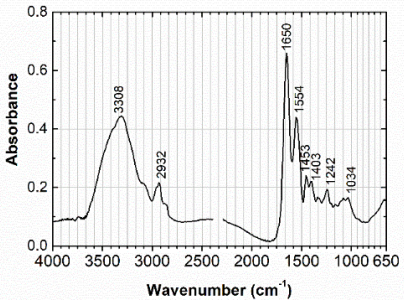
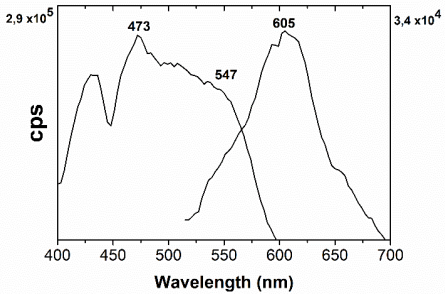
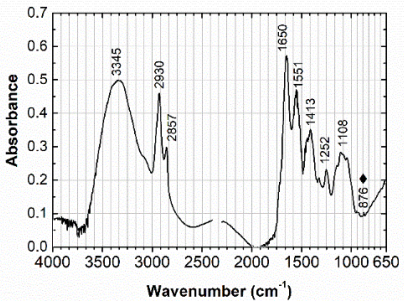
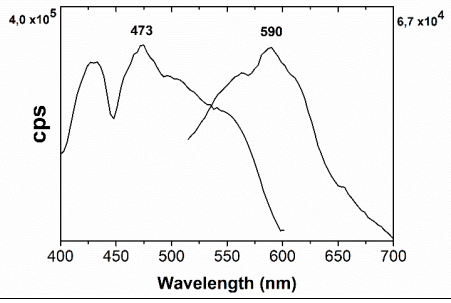
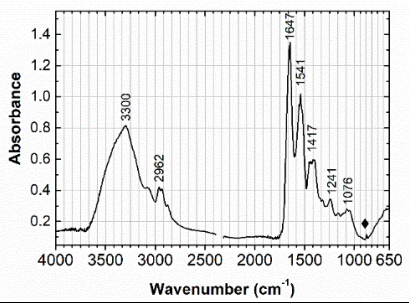
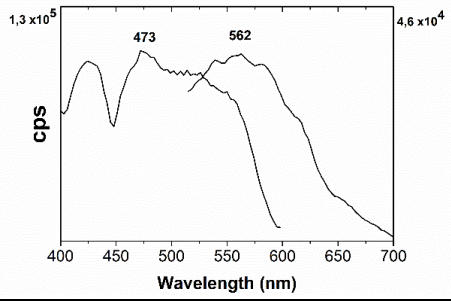
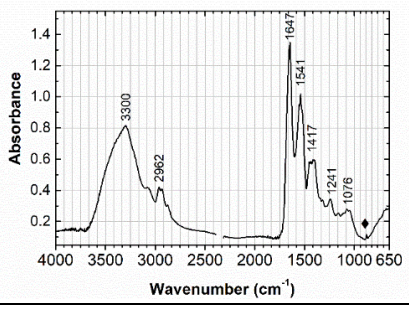
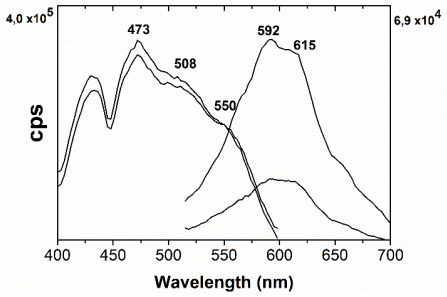
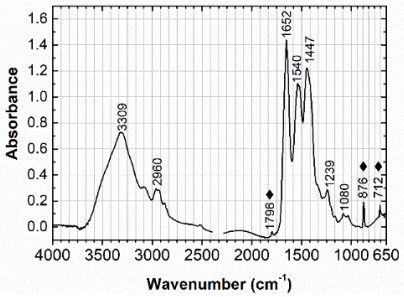
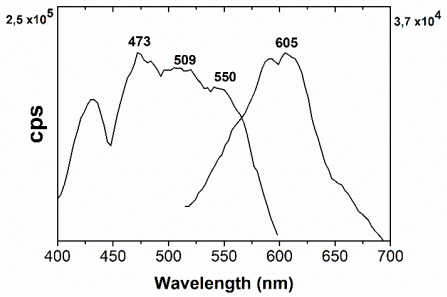
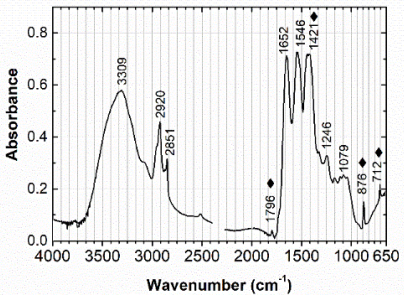
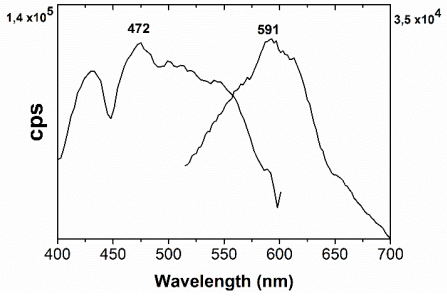
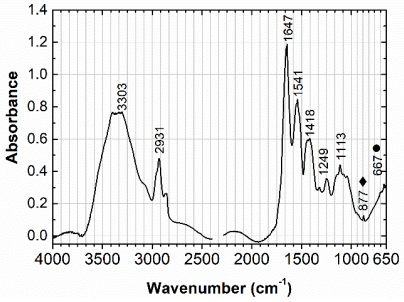
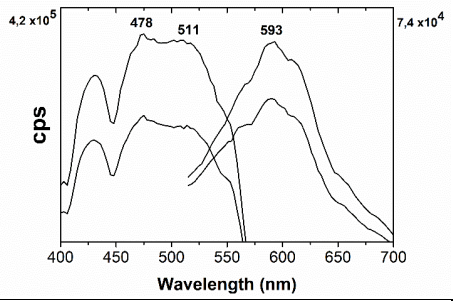
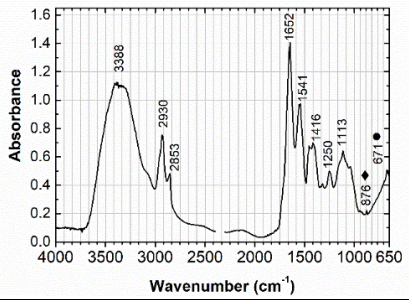
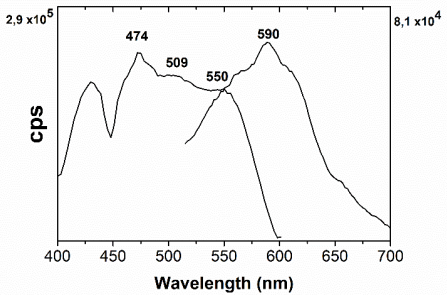
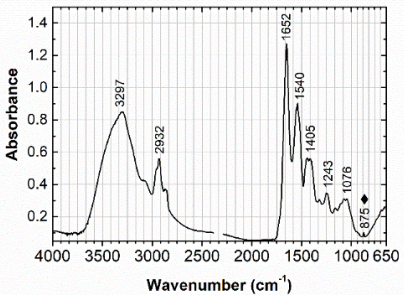


Figure A1.14. Typologies of the manuscripts found in Portuguese collections. Lac dye is found in 12th – 13th century manuscripts from the Alcobaça, Lorvão and Santa Cruz monasteries, while brazilwood is found the Ajuda Songbook and books of hours of Flemish and French production.

Table A1.5. *Group Lac 1* - Microspectrofluorimetry and infrared data of samples from lac dye in medieval manuscript illuminations (12th -13th c.). Abbreviations: Lorvão (Lv), Alcobaça (Alc), Santa Cruz (SC).

Manuscript	Folio	Fluorimetry	Infrared
SC 20 *Legendarium* Early 13th century	191 		
	197v 		
Lv 13 *Lectionarium Temporale* 13th century	21 $CaCO_3$ (◆)		
Alc. 412 *Homiliarum (copied by Iohannes Pecatoris)* 1257	10v  $CaCO_3$ (◆)		
	12 		

Lv 13 *Lectionarium Temporale* 13th century	30			
		>>$CaCO_3$ (◆)		
	44v dark			
		>>$CaCO_3$ (◆)		
Alc. 421 *Lectionarium Cisterciense* 12th - 13th century	202v			
		gypsum (●) $CaCO_3$ (◆)		
Alc. 446 *Etymologiae; De natura rerum ad Sisebutum by St Isidore of Seville* 13th century	96v			
		gypsum (●) $CaCO_3$ (◆)		
Lv 12 *Lectionarium Sanctorale* 13th century	17			
		$CaCO_3$ (◆)		

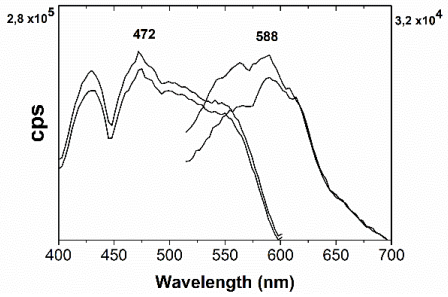
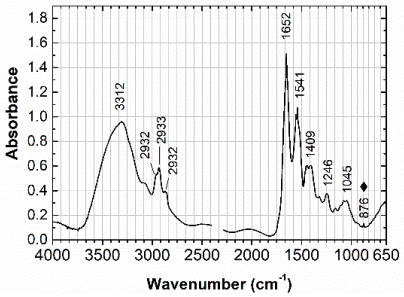
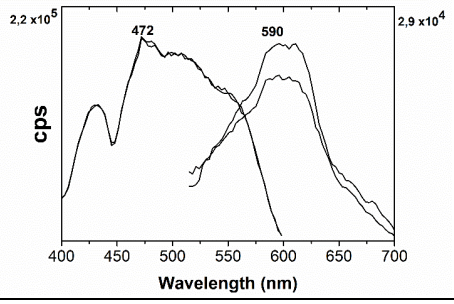
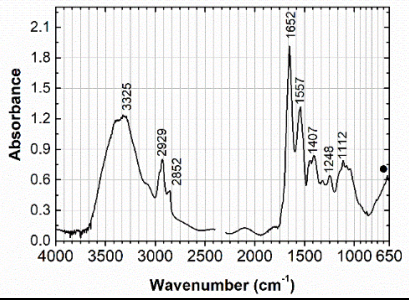
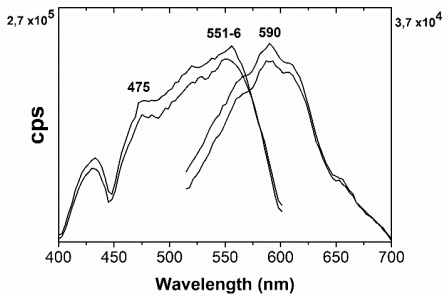
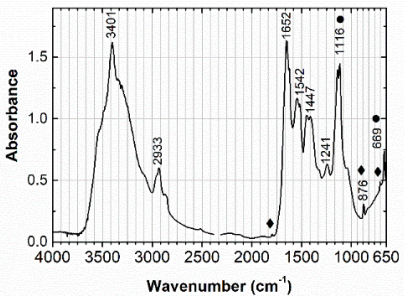
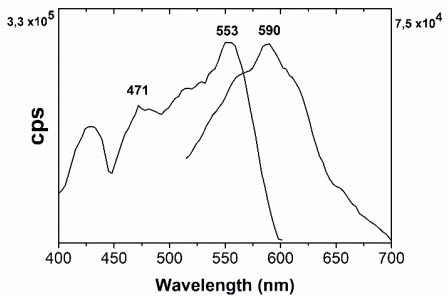
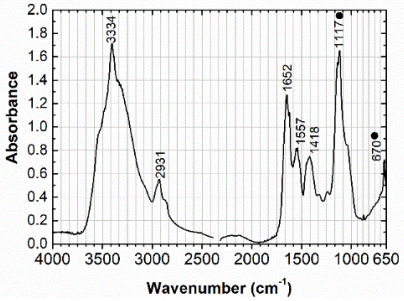
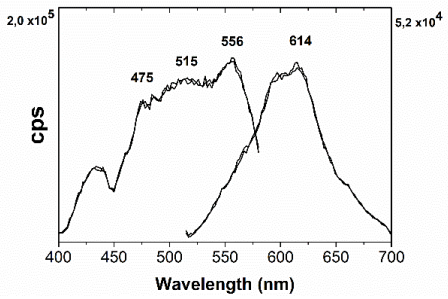
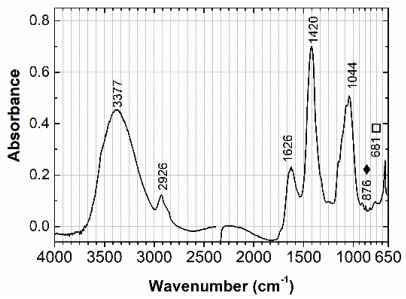
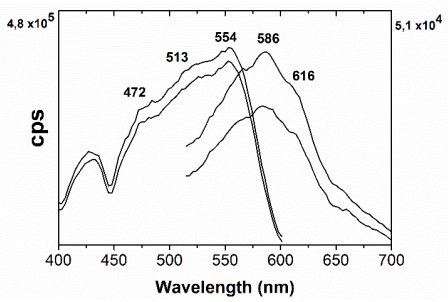
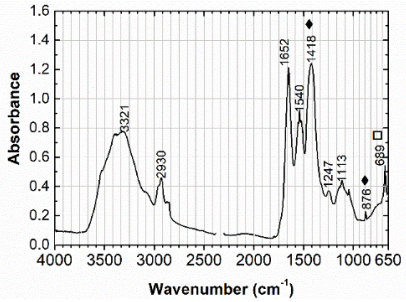
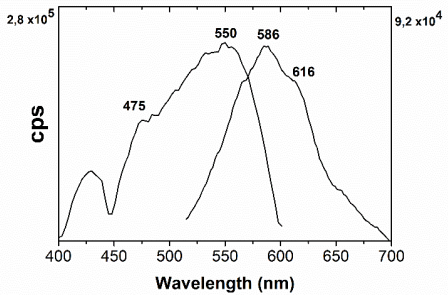
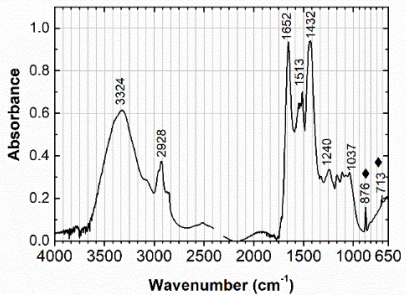
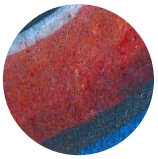
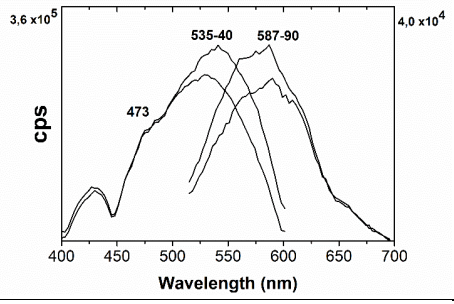
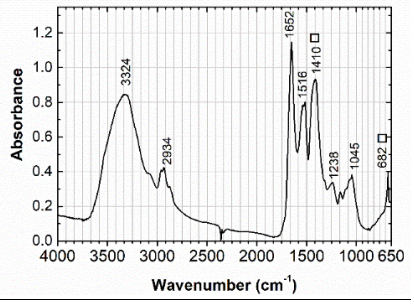
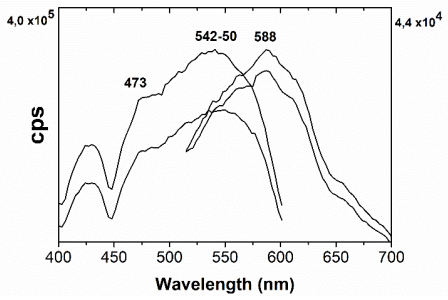
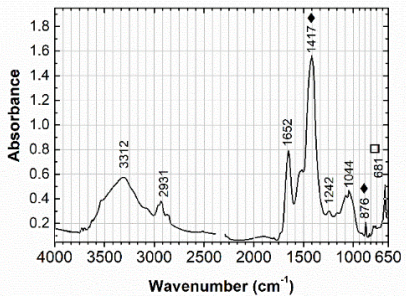
	94  $CaCO_3$ (◆)		
SC 21 *Legendarium* Early 13th century	19  gypsum (●)		

Table A1.6. *Group Lac 2* - Microspectrofluorimetry and infrared data of samples from lac dye in medieval manuscript illuminations (12th -13th c.). Abbreviations: Lorvão (Lv), Alcobaça (Alc), Santa Cruz (SC).

Manuscript	Folio	Fluorimetry	Infrared
Alc. 249 *Missal according to the Cistercian Rule* 13th century	109v  gypsum (●) $CaCO_3$ (◆)		
Alc. 419 *Lectionarium Cisterciense* 12th - 13th century	98  gypsum (●)		

Alc. 427 *Bible* 12th - 13th century	115v  $CaCO_3$ (◆) Lead white (□)		
Lv 15 *Gradual* 1201-50	16 $CaCO_3$ (◆) Lead white (□)		
	50 $CaCO_3$ (◆)		
Lv 50 *Enarrationes in Psalmos by Saint Augustine* 1183	1v  Lead white (□)		
	64v  $CaCO_3$ (◆) Lead white (□)		

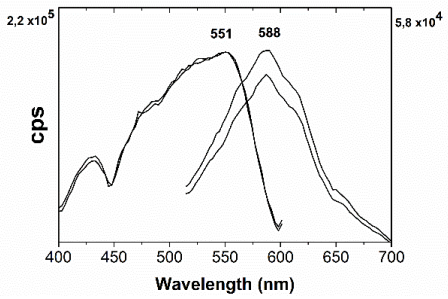
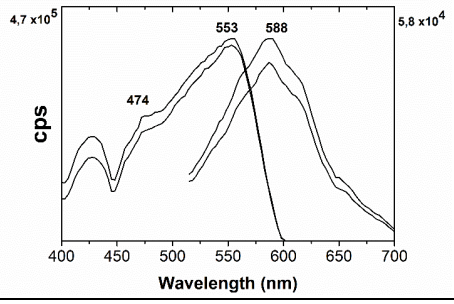
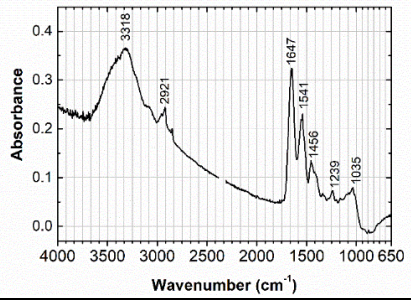
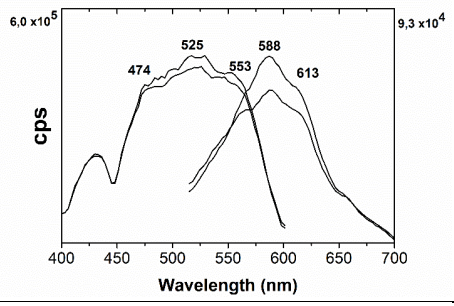
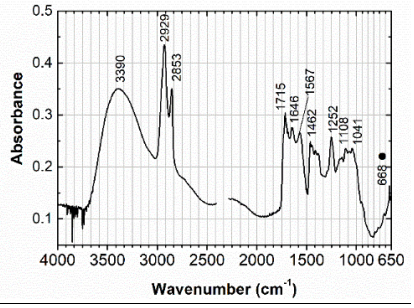
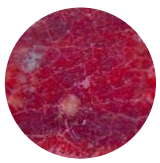
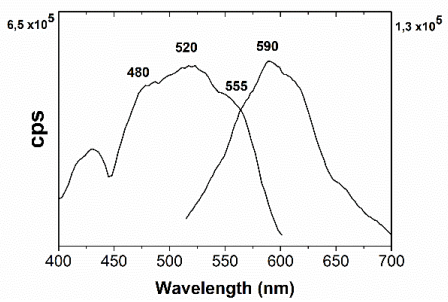
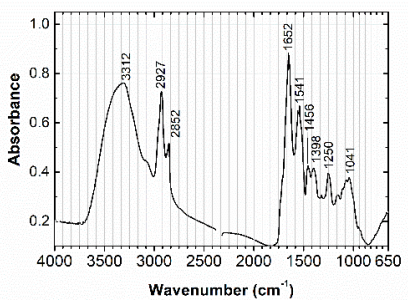
SC 20 *Legendarium* Early 13th century	78 		n.a.
	86  vermillion		

Table A1.7. ***Group Lac 3*** - Microspectrofluorimetry and infrared data of samples from lac dye in medieval manuscript illuminations (12th -13th c.). Abbreviations: Lorvão (Lv), Alcobaça (Alc), Santa Cruz (SC).

Manuscript	Folio	Fluorimetry	Infrared
Lv 5 *De avibus* by Hugh of Fouilloy 1183-4	6  gypsum (●)		
	73v 		

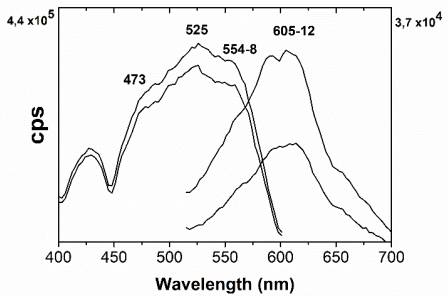
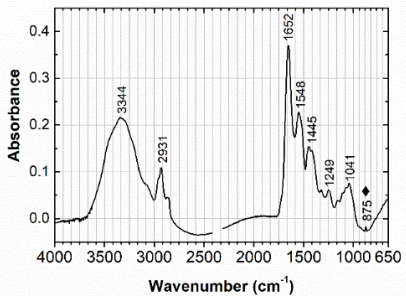
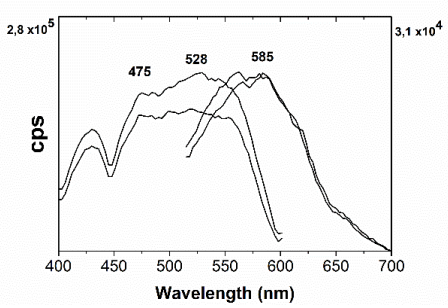
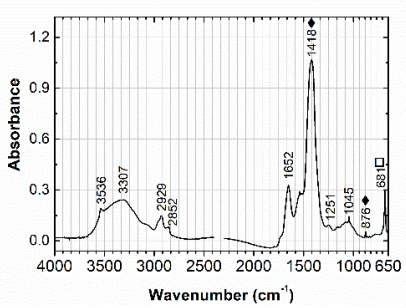
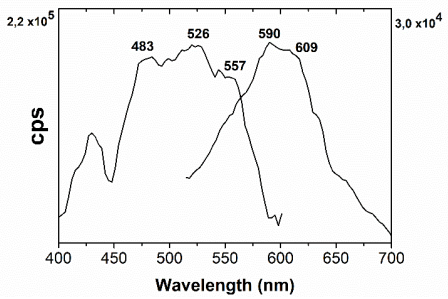
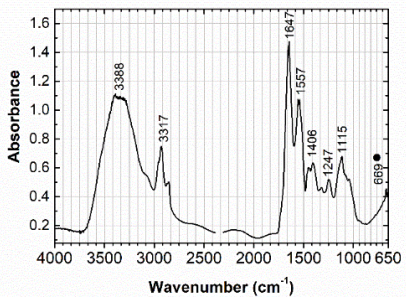
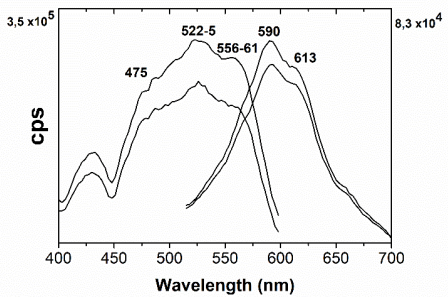
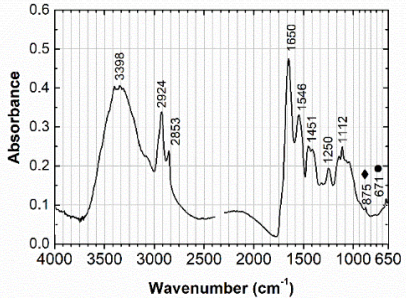
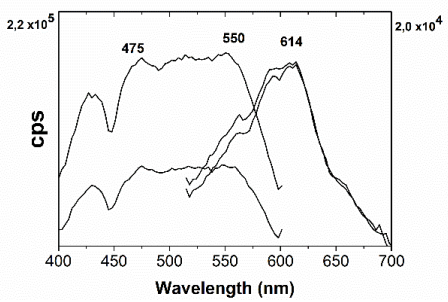
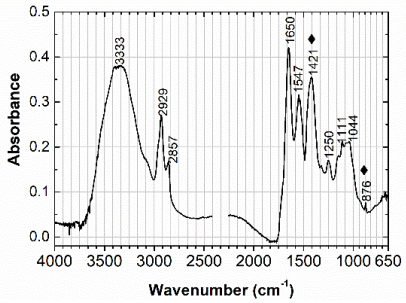
Lv 13 *Lectionarium Temporale* 13th century	44v light  $CaCO_3$ (◆)		
Lv 15 *Gradual* 1201-50	26  $CaCO_3$ (◆) Lead white (□)		
SC 21 *Legendarium* Early 13th century	2  gypsum (●)		
Alc. 247 *Commentary on the Apocalypse, by Biatus of Liébana* 12th century	21v  gypsum (●) $CaCO_3$ (◆)		
Alc. 421 *Lectionarium Cisterciense* 12th - 13th century	193v  $CaCO_3$ (◆)		

Table A1.8. ***Group Lac 4*** - Microspectrofluorimetry and infrared data of samples from lac dye in medieval manuscript illuminations (12th -13th c.). Abbreviations: Alcobaça (Alc).

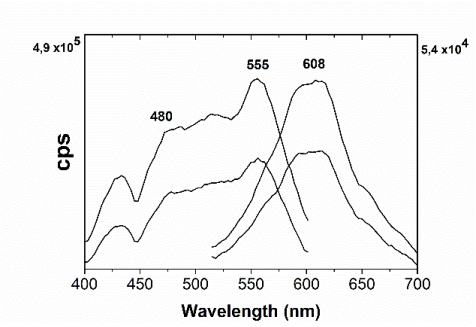
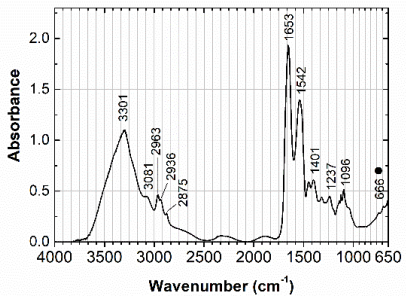
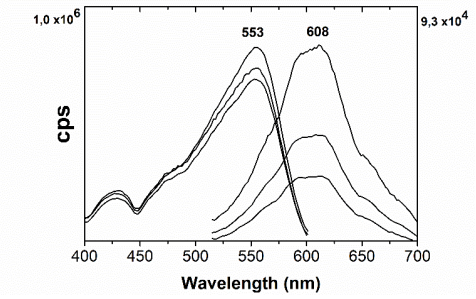
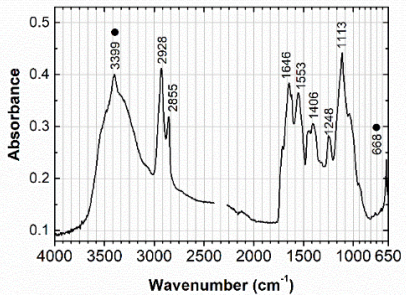
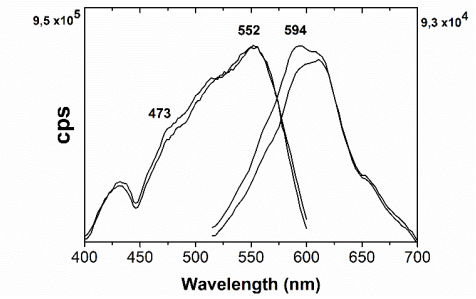
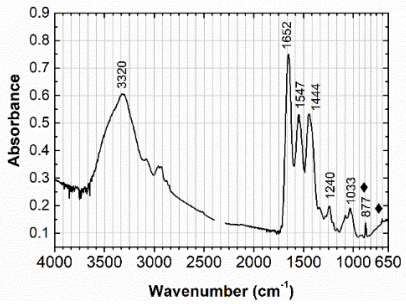
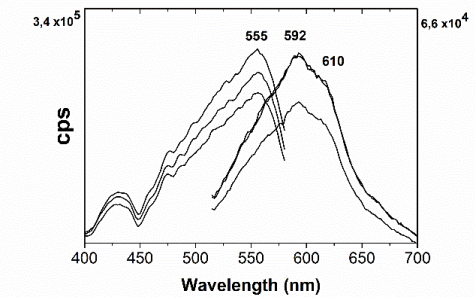
Manuscript	Folio	Fluorimetry	Infrared
Alc. 238 *Treaties, sentences and other texts (inc. De Avibus, Book of birds)* Late 12th century	203v gypsum (●)		
	206v gypsum (●)		
Alc. 347 *Sermones de verbis Domini; Sermones de verbis Apostoli* 12th - 13th century	3 lac  $CaCO_3$ (◆)		
	3 pink 		n.a.

Table A1.9. ***Group Lac 5, Bible SC 1*** - Microspectrofluorimetry and infrared data of samples from lac dye in medieval manuscript illuminations (12th -13th c.). Abbreviations: Santa Cruz (SC).

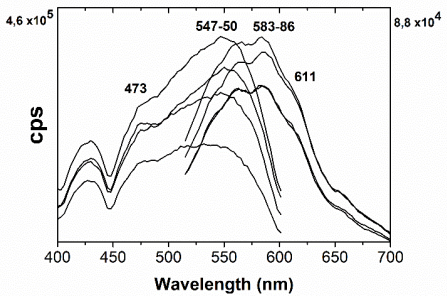
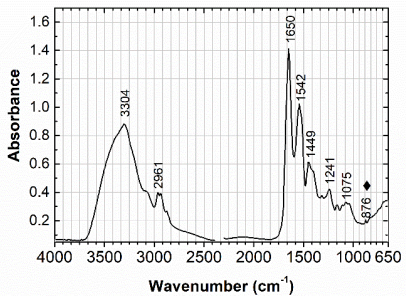
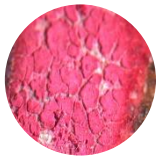
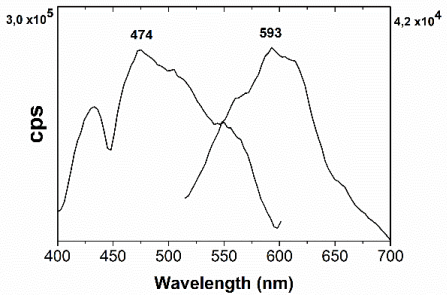
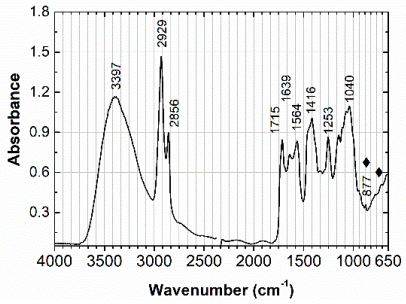
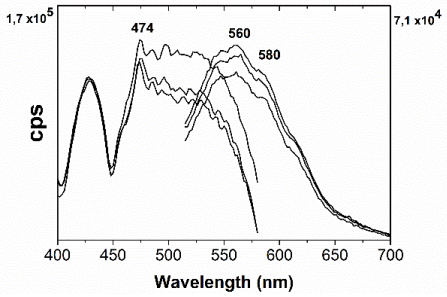
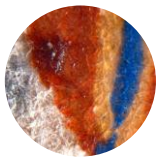
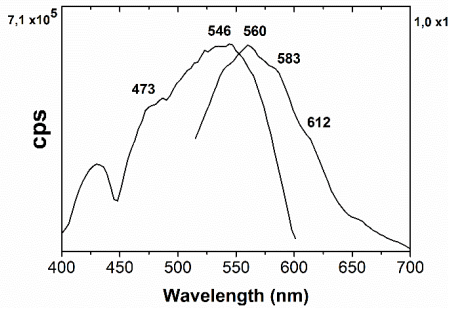
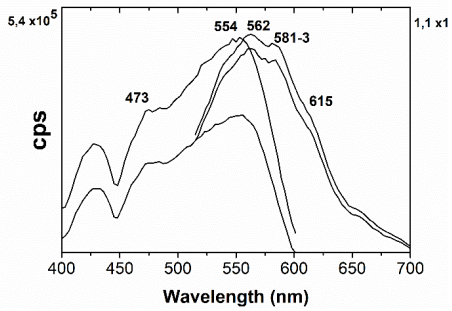
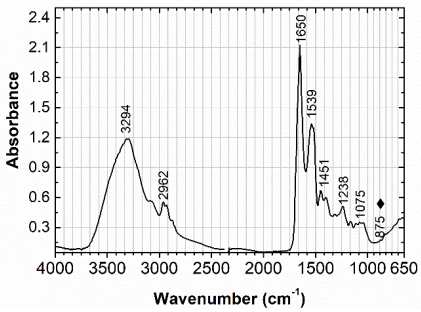
Manuscript	Folio	Fluorimetry	Infrared
SC 1 *Bible (Old Testament)* 1151-1200	2v  $CaCO_3$ (◆)		
	14v  $CaCO_3$ (◆)		
	24		n.a.
	37 		n.a.
	77  $CaCO_3$ (◆)		

Table A1.10. Microspectrofluorimetry and infrared data of lac dye reconstructions applied with glair as binder on filter paper.

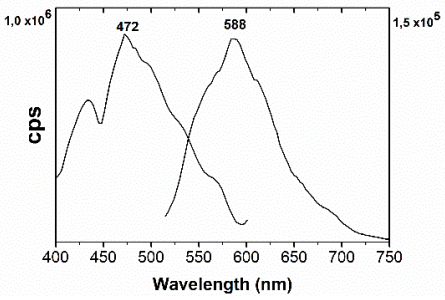
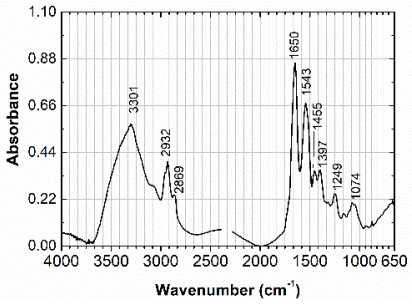
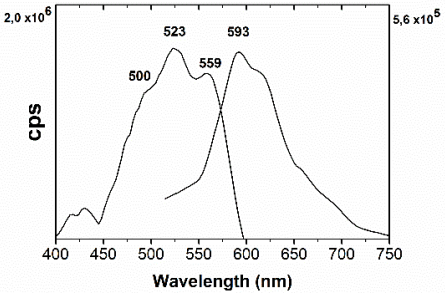
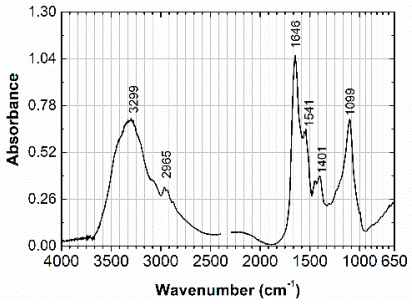
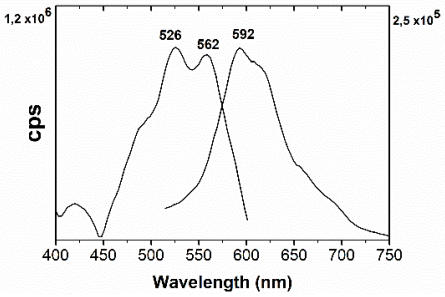
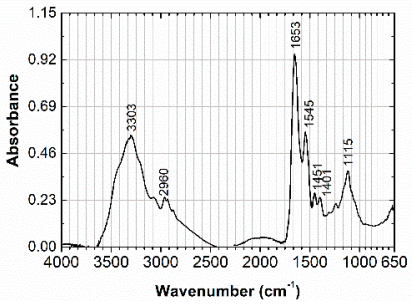
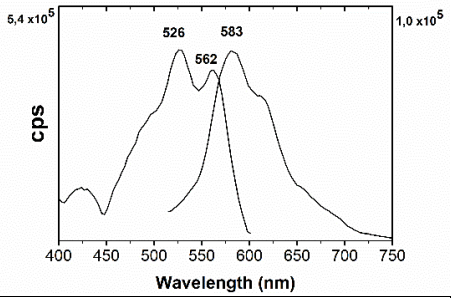
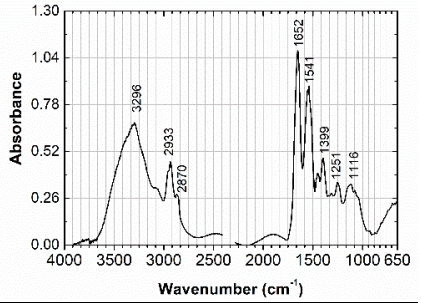
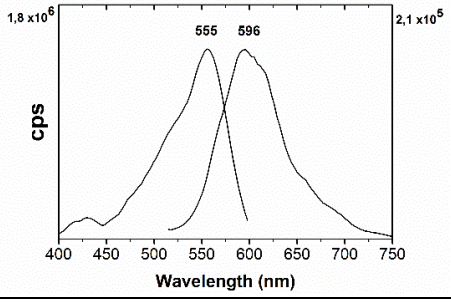
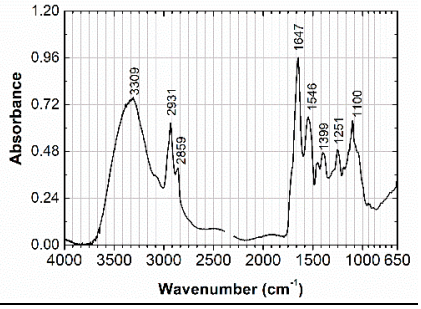
Treatise	Fluorimetry	Infrared
Ibn Badis		
Mappae Clavicula		
Bolognese 130		
Strasbourg		
Jean le Begue 309		

Table A1.11. Infrared bands of selected spectra of each group, compared with shellac and proteinaceous binder references.

Alc 446 ***Lac 1***	**Alc 249** ***Lac 2***	**Alc 247** ***Lac 3***	**Alc 238** ***Lac 4***	**Shellac**	**Proteinaceous binder**	**Assignments**
671 *w*	669 *m*	671 *w*	668 *m*	-	-	SO_4^{2-} bend
-	713 *w*	-	-	-	-	CO_3^{2-} bend
871 *w*	876 *w*	875 *w*	-	-	-	
1045 *sh*	1037 *sh*	1042 *sh*	1042 *sh*	1045	-	C-O str carboxylic acids
1113 *m*	1116 *s*	1112 *m*	1113 *s*	-	-	SO_4^{2-} str
1250 *m*	1241 *w*	1250 *m*	1248 *m*	1255	-	C-O str carboxylic acids
-	-	-	1406 *m*	1387	-	CH bend rings
1416 *m*	1415 *m*	1418 *m*	-	-	-	n.a.
1451 *m*	1447 *m*	1451 *m*	1451 *m*	-	1450	CN bend
-	-	-	-	1466	-	C=C str aromatic rings
1541 *s*	1542 *s*	1546 *s*	-	-	1541	Amide II CN str & NH bend
-	-	-	1553 *s*	1560	-	CN str & NH bend
-	-	-	-	1637	-	CO str
1652 *s*	1652 *s*	1650 *s*	1646 *s*	-	1651	Amide I CO str
-	-	-	1710 *sh*	1716	-	C=O str
-	1795 *w*	-	-	-	-	C=O str
2853 *m*	-	2853 *m*	2855 *s*	2858	-	CH str
-	2873 *sh*	-	-	-	2875	Sym methyl str
-	-	2924 *s*	2928 *s*	2931	-	CH str
2930 *s*	2933 *m*	-	-	-	2935	Asym methylene stretching
-	3401 *s*	3398 *br*	3399 *s*	-	-	water (gypsum)

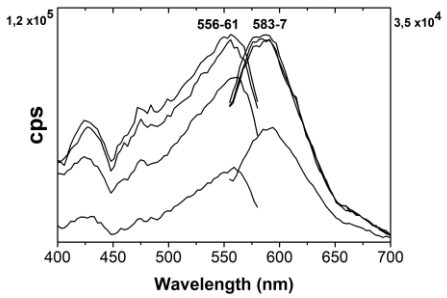
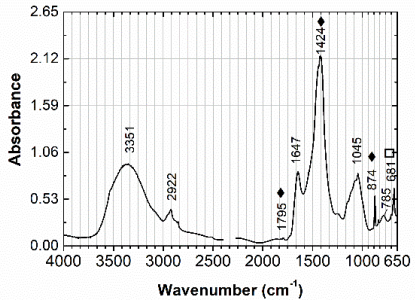
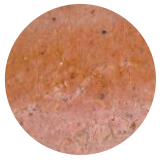
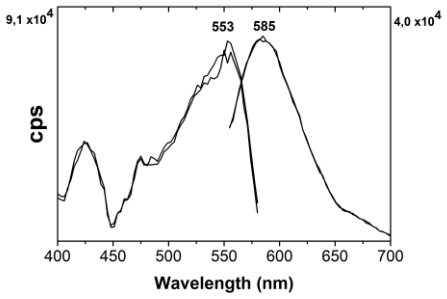
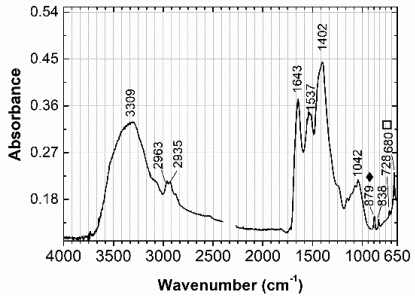
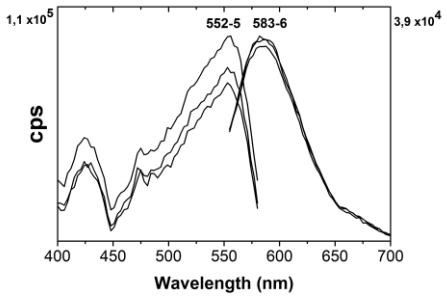
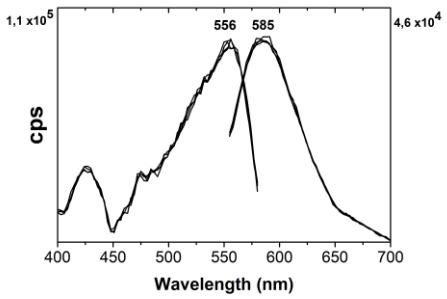
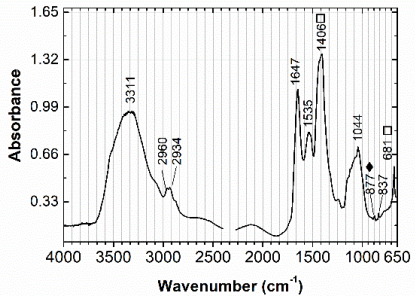
str stretching; *bend* bending; *sh* shoulder; *s* strong; *m* medium; *w* weak; *br* broad.

Bands corresponding to shellac resin (*orange*), to $CaCO_3$ (*dark grey*) and to $CaSO_4$ (*light grey*).

Table A1.12. Percentage of calcium carbonate calculated considering the ratio between the absorbance maxima of the bands at 1643 cm^{-1} and 876 cm^{-1}, $y=1.2x10^{-2}x + 7.6x10^{-3}$.

Manuscript		**Ratio**	**Percentage (%)**
Alc 412	fol. 12	0,034087	2,8
Alc. 421	fol. 202	0,045332	3,7
Alc. 446	fol. 96v	0,031948	2,7
Lv 12	fol.94	0,02876	2,4
Lv 13	fol. 21v	0,032302	2,7
	fol. 30	0,145518	12,1
	fol.44v	0,147585	12,3
SC 20	fol.191	0,014746	1,2
	fol.197	0,028026	2,3
SC 21	fol19	0,02878	2,4

Table A1.13. ***Group BW 1*** - Microspectrofluorimetry and infrared data of samples from brazilwood in medieval manuscript illuminations (end of 13th -15th c.)[83].

Manuscript	Folio	Fluorimetry	Infrared
Ajuda Songbook *Songbook in Galician-Portuguese* End of 13th century – beginning of the 14th century	4  $CaCO_3$ (◆) Lead white (□)		
	17  $CaCO_3$ (◆) Lead white (□)		
	21 		n.a.
	59  $CaCO_3$ (◆) Lead white (□)		

[83] The attributions of the books of hours present in this work were conducted by Ana Lemos and coworkers. For more information please see: Lemos A, et al. Regards croisés des historiens de l'art et des chimistes sur deux livres d'heures de la Bibliothèque Nationale Du Portugal, Les Mss. IL15 et IL19. 2015; Lemos A, et al. O Cofre nº 24: um livro de horas do Palácio Nacional de Mafra, caso de estudo, 2015; Melo et al. Segredos descobertas nos Livros de Horas: história, materiais e técnicas, 2015; Melo et al. O que nos dizem os materiais da cor sobre os livros de horas do Palácio Nacional de Mafra?. 2012.

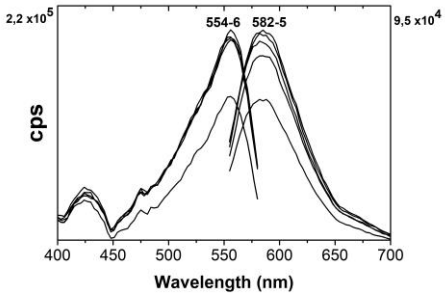
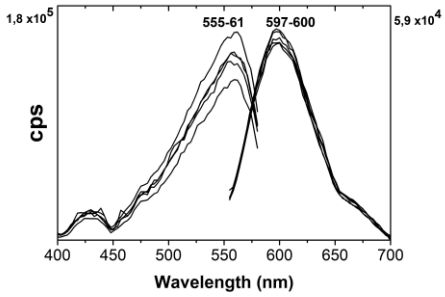
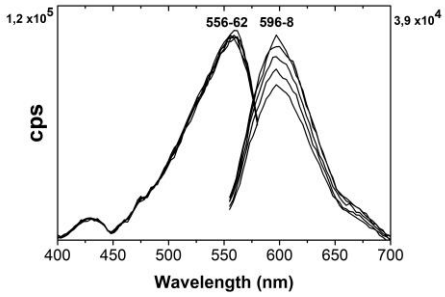
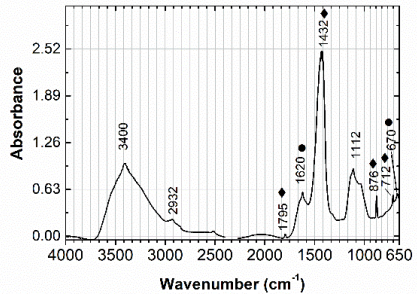
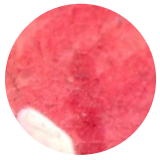
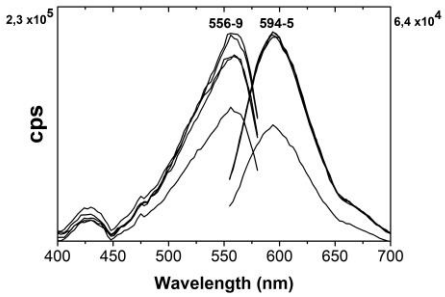
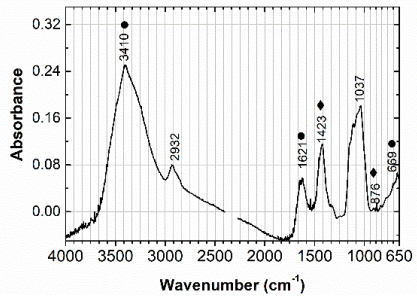
IL 15 *Book of Hours of Flemish production, Bruges* ca. 1450	60 		n.a.

Table A1.14. *Group BW 2* - Microspectrofluorimetry and infrared data of samples from brazilwood in medieval manuscript illuminations (14th -15th c.).

Manuscript	Folio	Fluorimetry	Infrared
IL 15 *Book of Hours of Flemish production, Bruges* *ca.* 1450	15 		n.a.
IL 19 *Book of Hours of French production, Paris* 1420-30	91  gypsum (●) $CaCO_3$ (◆)		
IL 21 *Book of Hours of French production, Troyes* 1460-70	88  gypsum (●) $CaCO_3$ (◆)		

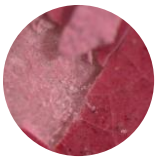
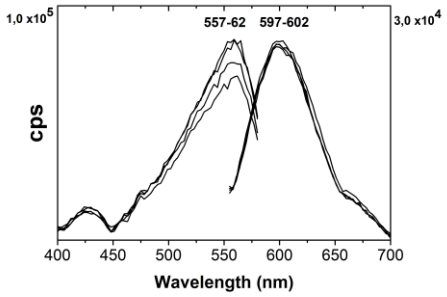
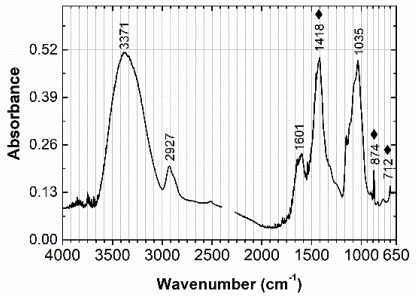
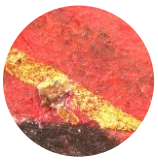
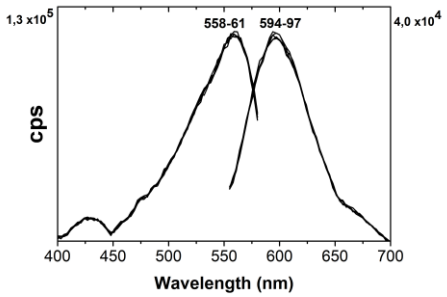
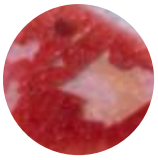
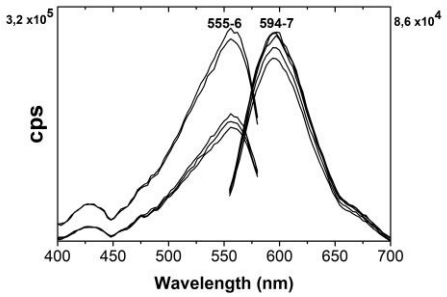
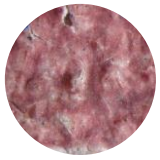
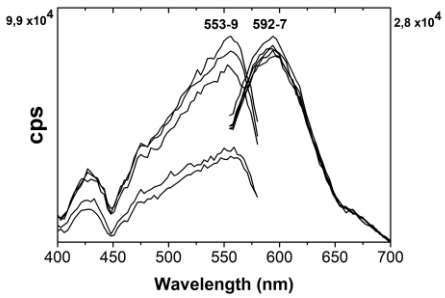
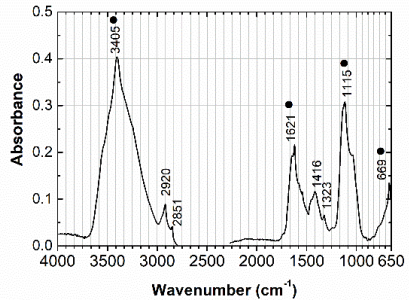
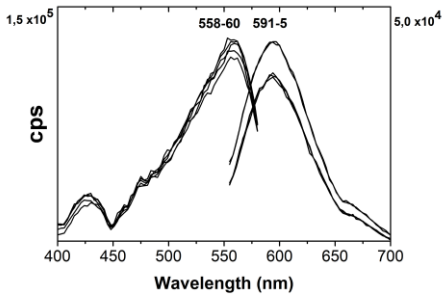
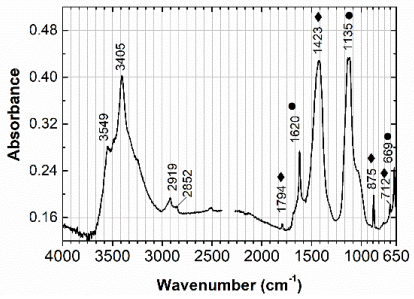
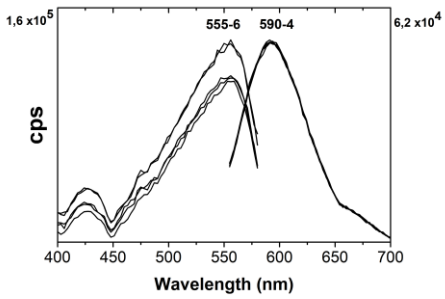
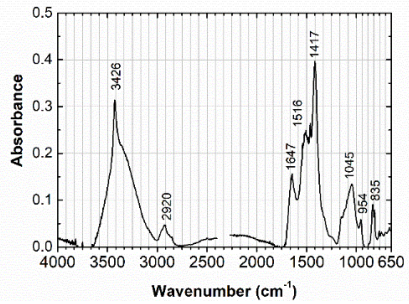
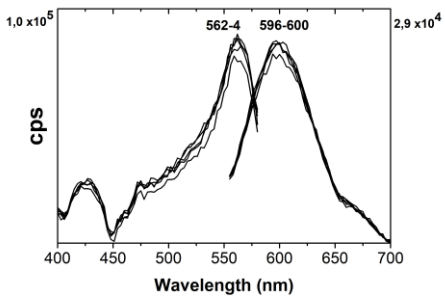
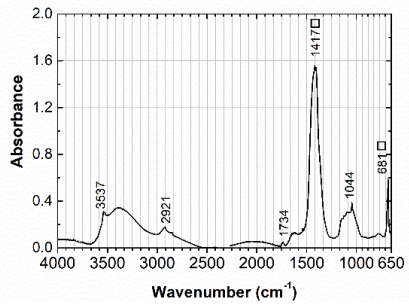
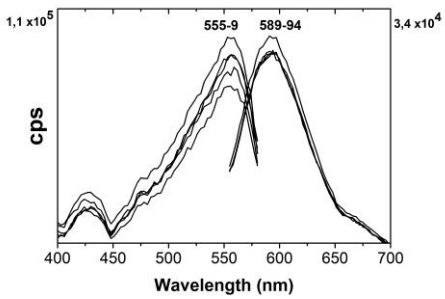
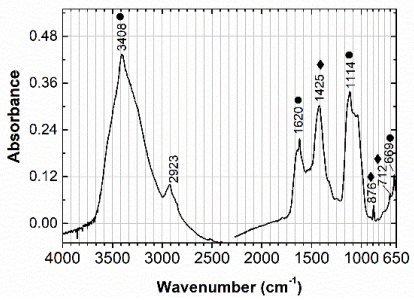
IL 42 *Book of Hours of French production, Rouen* *ca.* 1470	9  $CaCO_3$ (◆)		
	85 		n.a.
	113 		n.a.

Table A1.15. ***Group BW 3*** - Microspectrofluorimetry and infrared data of samples from brazilwood in medieval manuscript illuminations (14th -15th c.).

Manuscript	Folio	Fluorimetry	Infrared
Cofre nº 22 *Book of Hours of French production, Paris* 1400-20	76v  gypsum (●)		
Cofre nº 24 *Book of Hours of French production, Autun* 1420 / 1470	60  gypsum (●) $CaCO_3$ (◆)		
IL 15 *Book of Hours of Flemish production, Bruges* *ca.* 1450	66 		
IL 19 *Book of Hours of French production, Paris* 1420-30	21  Lead white (□)		
IL 42 *Book of Hours of French production, Rouen* *ca.* 1470	23  gypsum (●) $CaCO_3$ (◆)		

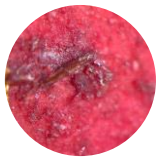
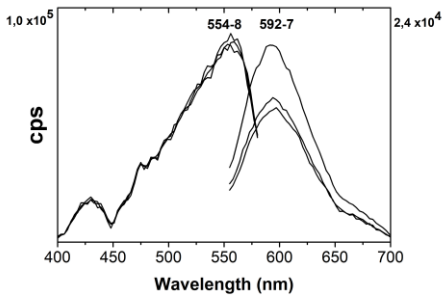
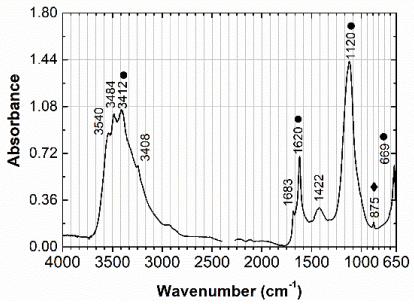
	133  gypsum (●) $CaCO_3$ (◆)		

Table A1. 16. ***The Winter Breviary, Alc. 54*** - Microspectrofluorimetry and infrared data of samples from brazilwood in medieval manuscript illuminations (14th-15th c.).

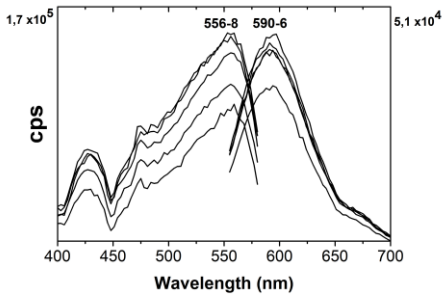
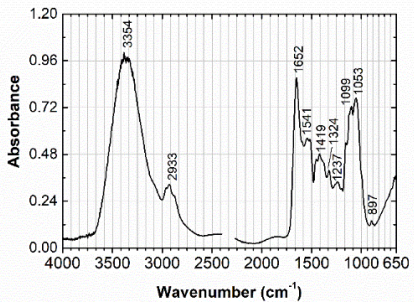
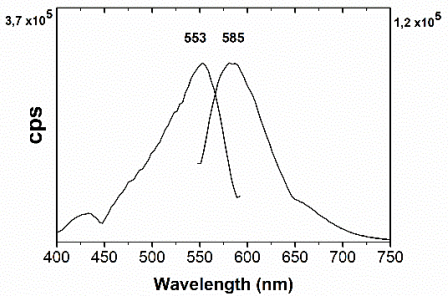
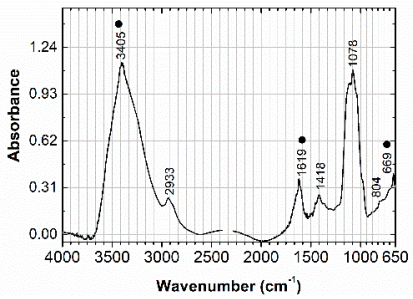
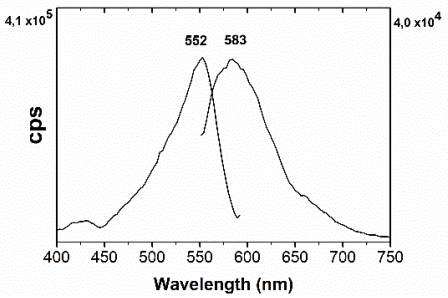
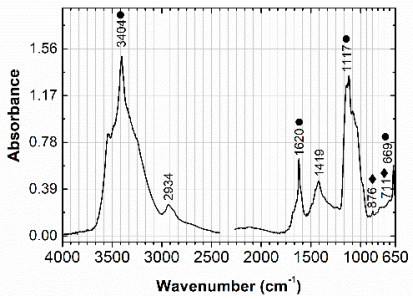
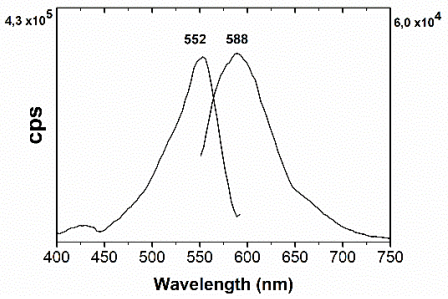
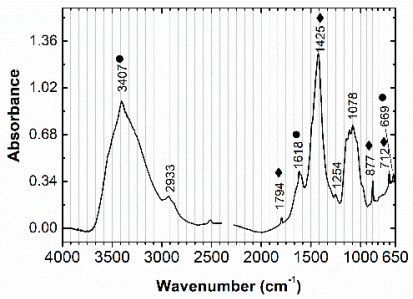
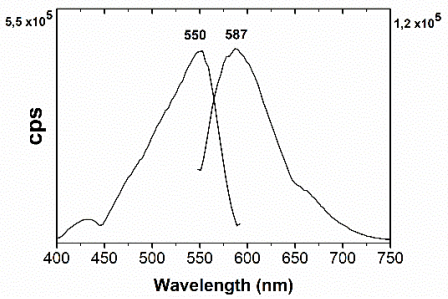
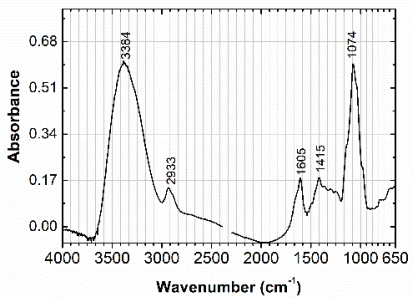
Manuscript	Folio	Fluorimetry	Infrared
Alc. 54 *Winter Breviary of the Alcobaça Monastery* 14th century	92 		

Table A1.17. Microspectrofluorimetry and infrared data of brazilwood reconstructions based on the '*The book of all color paints*', applied with gum arabic as binder on filter paper.

Treatise	Fluorimetry	Infrared
Recipe 8  gypsum (●)		
Recipe 9  gypsum (●) $CaCO_3$ (◆)		
Recipe 27  gypsum (●) $CaCO_3$ (◆)		
Recipe 44 		

Areas of analysis

For µ-sampling (●)

BRAZILWOOD

Figure A1.15. Ajuda Songbook, folio 4, © Ajuda Library.

Figure A1.16. Ajuda Songbook, folio 17, © Ajuda Library.

Figure A1.17. Ajuda Songbook, folio 21, © Ajuda Library.

Figure A1.18. Ajuda Songbook, folio 59, © Ajuda Library.

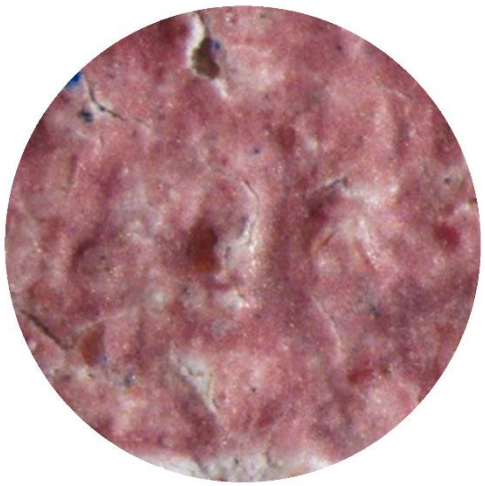


Figure A1.19. Book of Hours Cofre nº 22, folio 76v, © PNMAF.

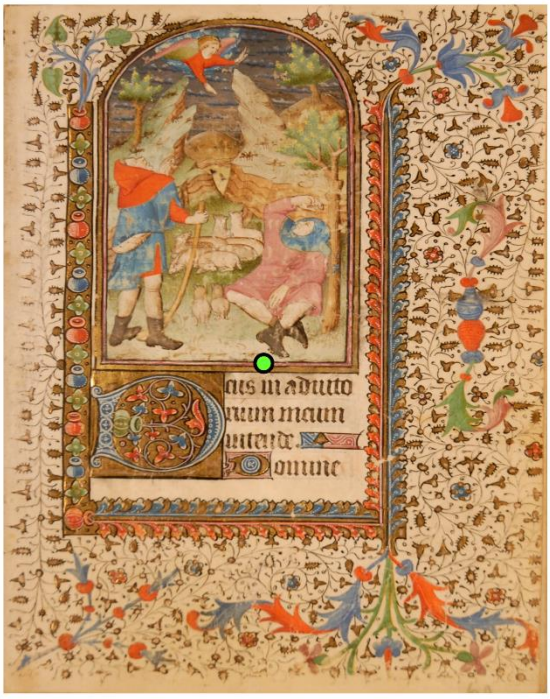


Figure A1.20. Book of Hours Cofre nº 24, folio 60, © PNMAF.

Figure A1.21. Book of Hours IL 15, folio 15, © BNP.

Figure A1.22. Book of Hours IL 15, folio 60, © BNP.

Figure A1.23. Book of Hours IL 15, folio 66, © BNP.

Figure A1.24. Book of Hours IL 19, folio 21, © BNP.

Figure A1.25. Book of Hours IL 19, folio 91, © BNP.

Figure A1.26. Book of Hours IL 21, folio 88, © BNP.

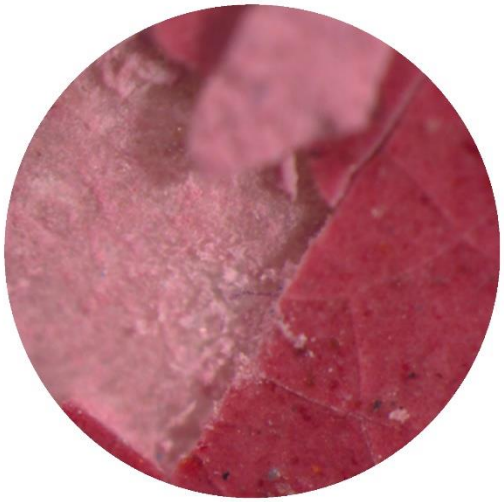


Figure A1.27. Book of Hours IL 42, folio 9, © BNP.

Figure A1.28. Book of Hours IL 42, folio 23, © BNP.

Figure A1.29. Book of Hours IL 42, folio 85, © BNP.

Figure A1.30. Book of Hours IL 42, folio 113 © BNP.

Figure A1.31. Book of Hours IL 42, folio 133, © BNP.

COCHINEAL[84]

Figure A1.32. Weaver's workbasket, Chancay 11th – 15th centuries, photograph © Museum of Fine Arts, Boston.

[84] Andean textiles photographs © Museum of Fine Arts, Boston.

Figure A1.33. Tunic, Chancay 11th – 15th centuries, photograph © Museum of Fine Arts, Boston.

Figure A1.34. Tapestry, Lambayeque 11th – 15th centuries, photograph © Museum of Fine Arts, Boston.

LAC DYE

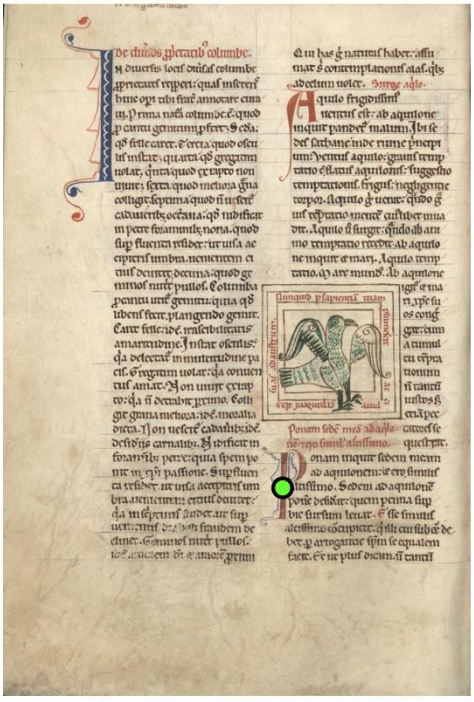


Figure A1.35. Alcobaça 238, folio 206v, © BNP.

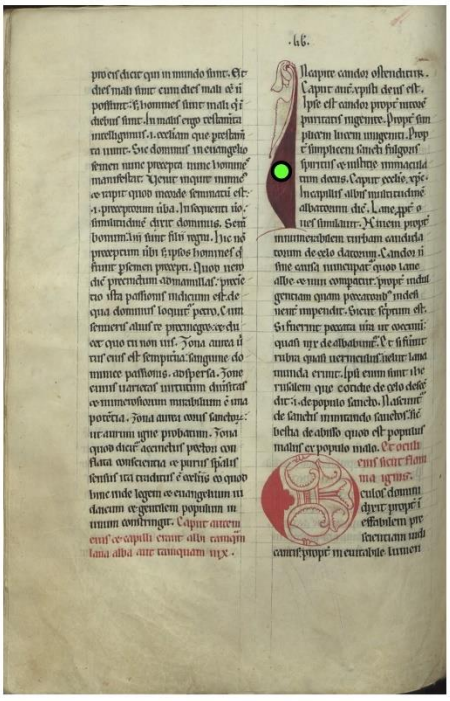


Figure A1.36. Alcobaça 247, folio 21v, © BNP.

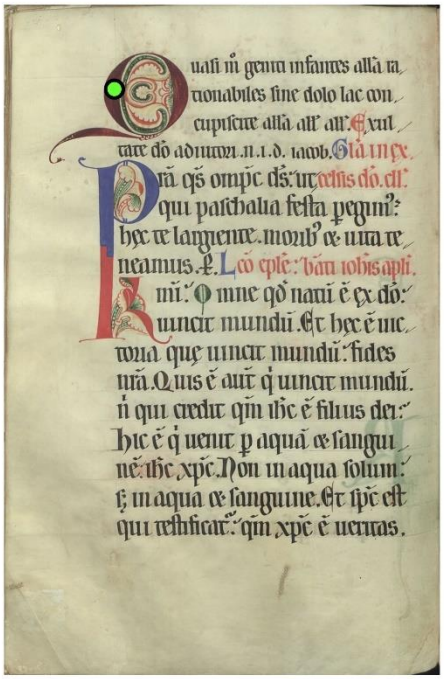


Figure A1.37. Alcobaça 249, folio 109v, © BNP.

Figure A1.38. Alcobaça 347, folio 3, © BNP.

Figure A1.39. Alcobaça 412, folio 10v, © BNP.

Figure A1.40. Alcobaça 419, folio 98, © BNP.

Figure A1.41. Alcobaça 421, folio 193v, © BNP.

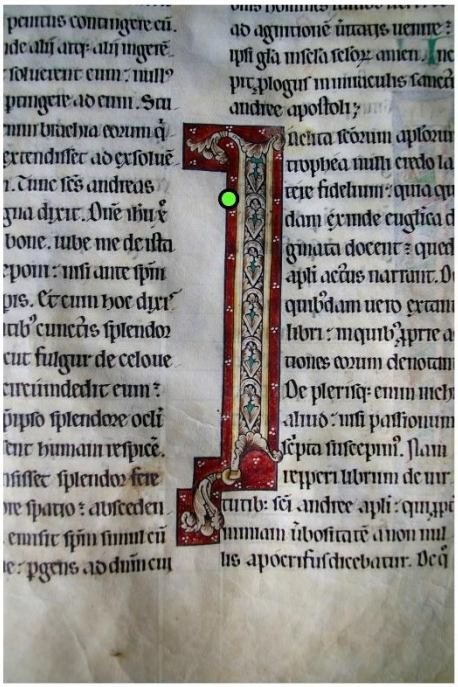


Figure A1.42. Alcobaça 421, folio 202, © BNP.

Figure A1.43. Alcobaça 446, folio 96v, © BNP.

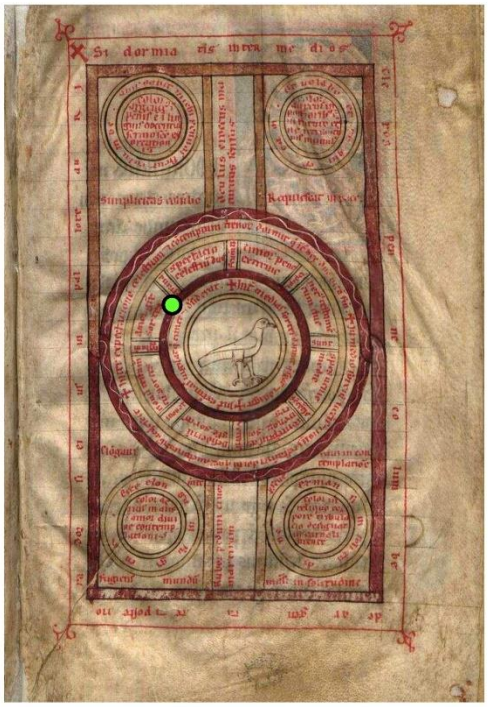


Figure A1.44. Lorvão 5, folio 6, © BPMP.

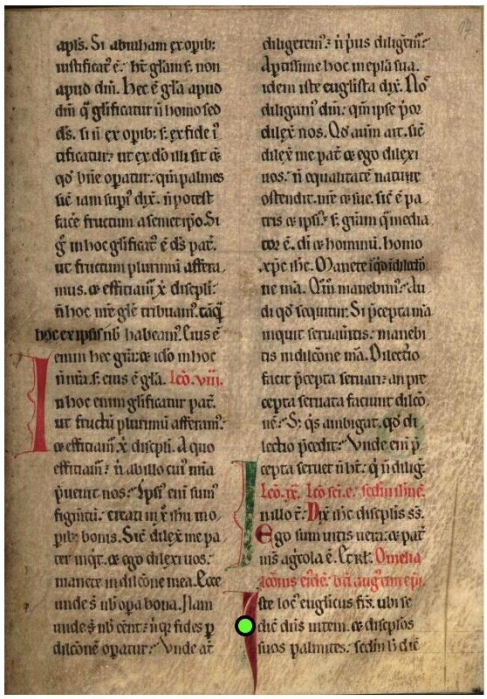


Figure A1.45. Lorvão 12, folio 17, © BPMP.

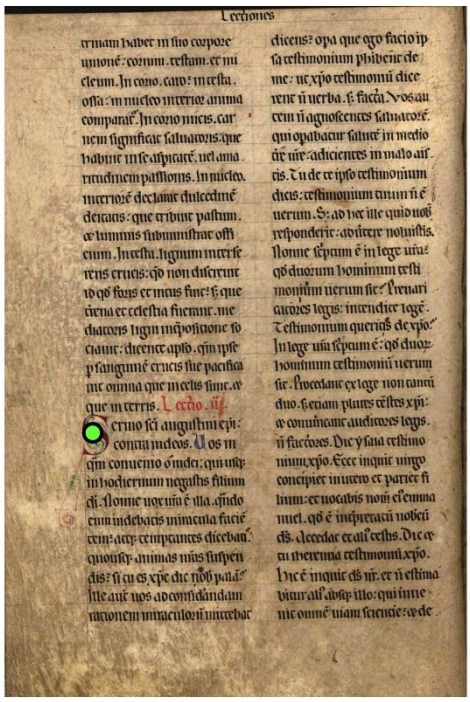


Figure A1.46. Lorvão 13, folio 21v, © BPMP.

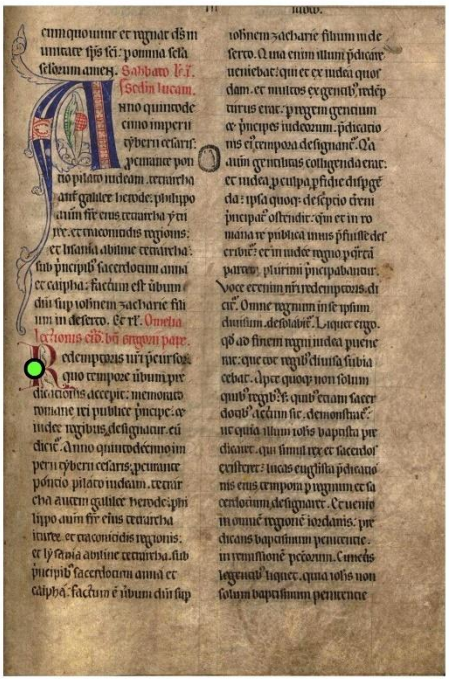


Figure A1.47. Lorvão 13, folio 30, © BPMP.

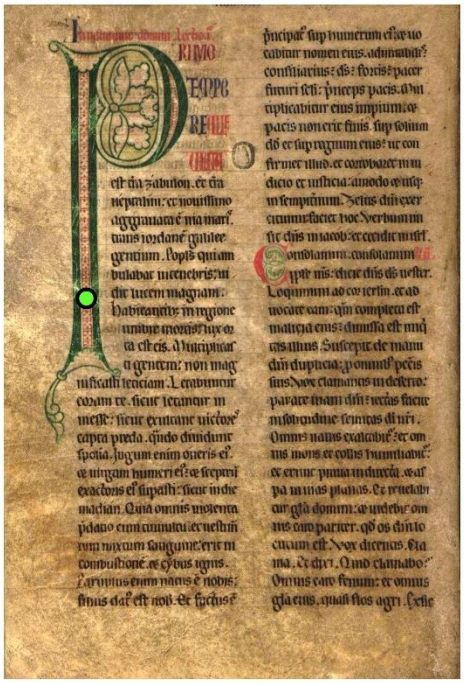


Figure A1.48. Lorvão 13, folio 44v, © BPMP.

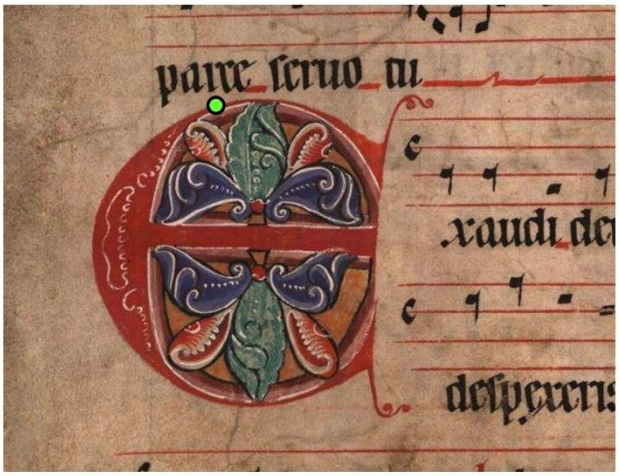


Figure A1.49. Lorvão 15, folio 26, © BPMP.

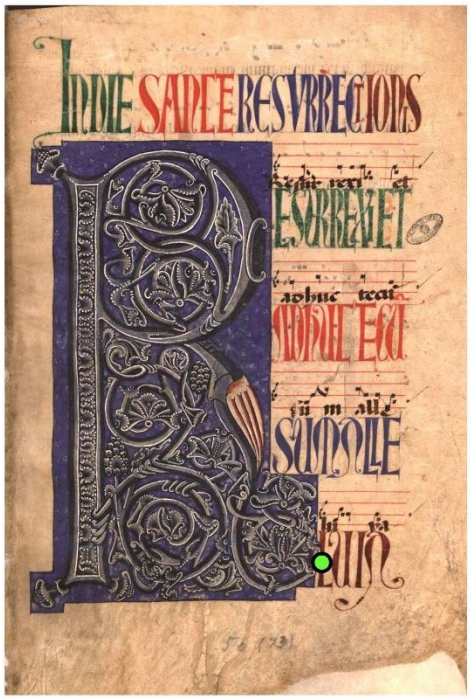


Figure A1.50. Lorvão 15, folio 50, © BPMP.

Figure A1.51. Lorvão 50, folio 1v, © BPMP.

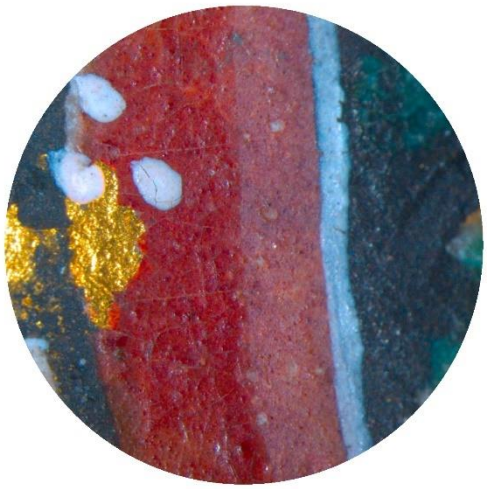


Figure A1.52. Lorvão 50, folio 64v, © BPMP.

Figure A1.53. Santa Cruz 1, folio 2v, © ANTT.

Figure A1.54. Santa Cruz 1, folio 77, © ANTT.

Figure A1.55. Santa Cruz 21, folio 2, © ANTT.

Figure A1.56. Santa Cruz 21, folio 19, © ANTT.

Appendix 2. Red and puple organic colorants in medieval manuscripts

A2.1. Brazilwood in the Ajuda Songbook

Previously we mentioned the use of pink in the Songbook.[85] The pink is one of the main colors found in the Songbook, where it is applied as a light pink in architecture as well as clothing. It is also found shaded with a protein varnish that saturates its color, producing a darker pink. Additives were used to alter its appearance further: gypsum was added to render it opaque, while a lighter hue was created by admixing lead white. The darker pink is found in the clothing worn by the noblemen, while the lighter pink was identified in the architecture. In all the illuminations analyzed, in both light and dark pink colors, FORS and microspectrofluorimetry have detected the brazilwood chromophore and comparison with historical paint reconstructions showed a good match with recipe 9 from 'the book of all color paints', **Figure A2.1**

Table A2.1. Absorption (−log*R*), excitation and emission maxima for the pink from the Ajuda Songbook compared with a brazilwood paint reference

	λabs (nm)	λexc (nm)	λem (nm)
Ajuda Songbook, pink	554	552-558	565-568 580 (sh)
Brazilwood paint[1]	553	553	568

1 – Recipe 9, Exp. 5, *'The book of all color paints'* [33].

The absorption (554 nm) and excitation (552–558 nm) correlates well with the 553 nm maxima for the paint reconstruction. Similarly, the emission maxima at 565–568 nm correlates with the 568 nm value in the brazilwood paint reference, see **Figure A2.1** and **Table A2.1**. Concerning the Raman analysis, it was not possible to record a Raman spectrum (owing to the high fluorescence of the paints), and SERS analysis proved unsuccessful, with and without the HF treatment.

When applying the chemometric approach developed in **Chapter 2**, it is visible that the pink from the Ajuda Songbook falls within the brazilwood cluster. The data presented in **Figure A2.2** was generated with data from artworks, from textiles and manuscripts, from the 11th – 15th c., described in **Chapter 3**.

[85] The of these results relatve to the Ajuda Songbook have been published and can be consulted at Melo MJ, Nabais P, Guimarães M, Araújo R, Castro R, Oliveira MC, Whitworth I. Organic dyes in illuminated manuscripts: an unique cultural and historic record. Phil Trans R Soc A. 2016;374(2082):20160050.

The closest samples to the Ajuda Songbook are those belonging to a winter Breviary, Alc. 54, produced in the *scriptorium* of the Monastery of Santa Maria of Alcobaça. The original *corpus* is dated from the 14th century, with two 15th c. additions. Both the absorption (555 nm) and the excitation (554-8 nm), as well as the emission (585 nm with a shoulder at 565 nm) maxima of the pinks from the winter Breviary correlate well with those from the Ajuda Songbook and with the paint from recipe 9 of the 'the book of all color paints'.

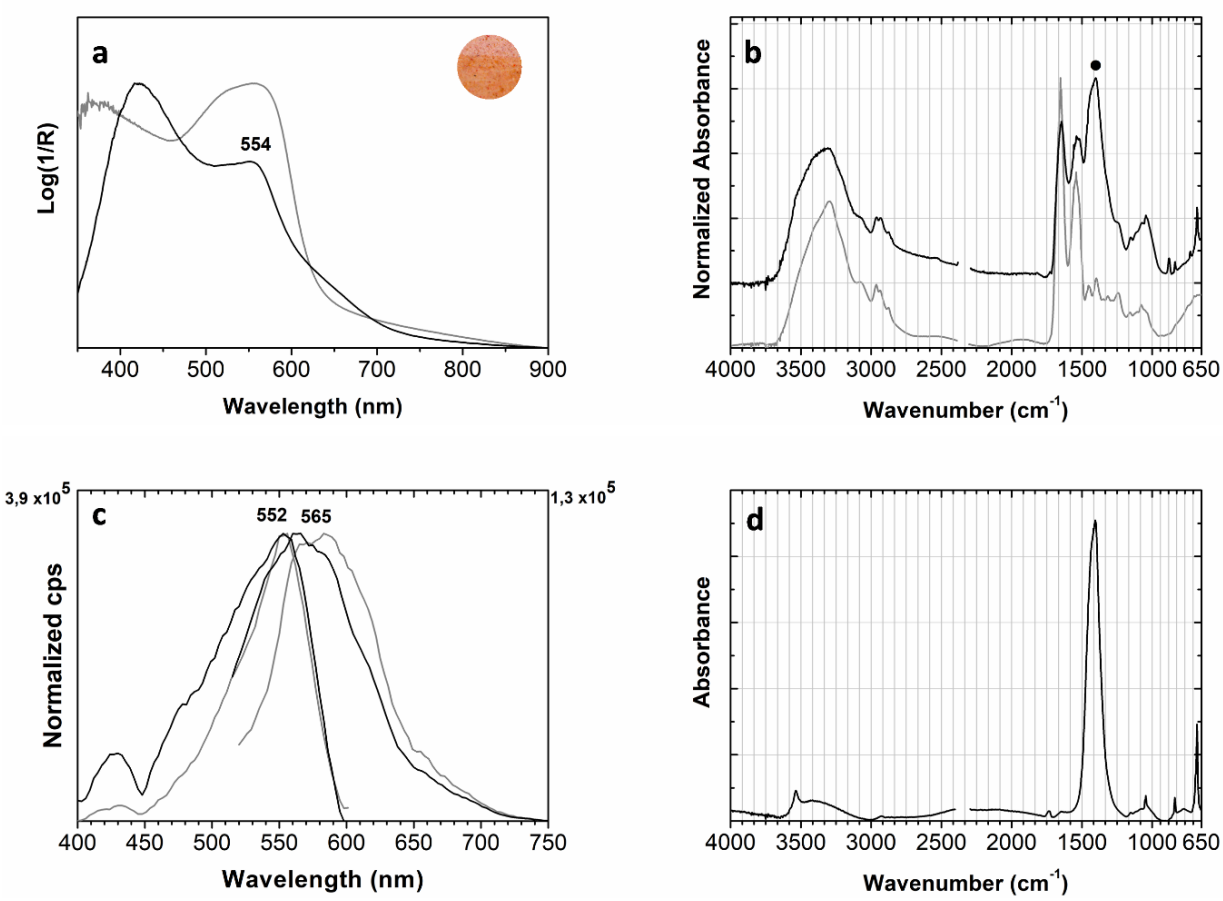


Figure A2.1. Brazilwood in architectural elements and vestments, as proved by its molecular spectra. Spectra for brazilwood paint reference (*grey*, '*The book of all color paints*', recipe 9) and pink in the noble's vestments, fol. 59 (*black*): (*a*) apparent absorption (-log*R*); (*b*) infrared; characteristic peaks for lead white (●), reference in (*d*), with a protein binder (*grey*); (*c*) excitation and emission spectra.

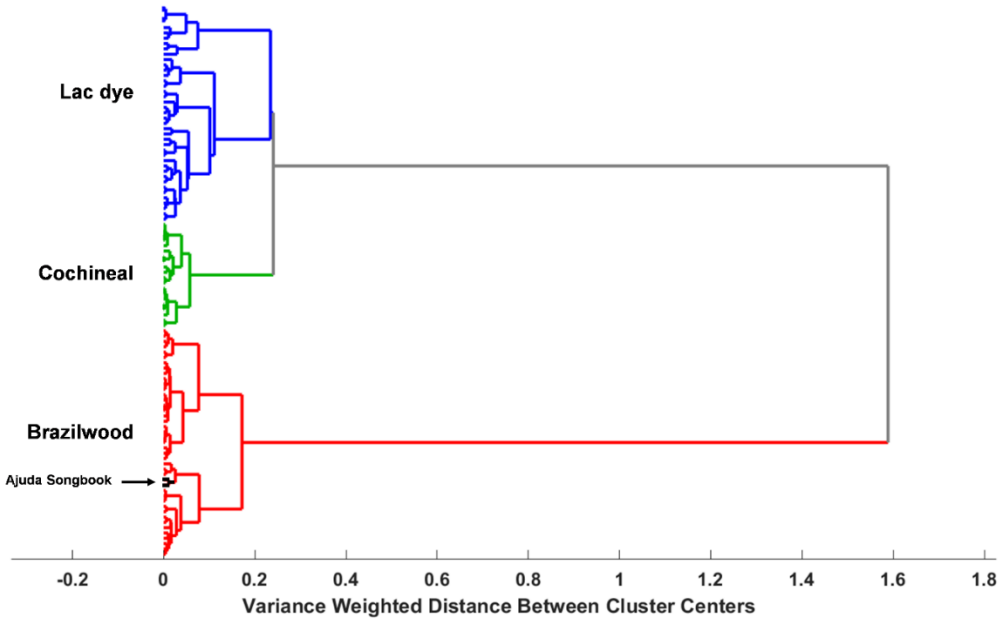


Figure A2.2. Dendrogram generated by HCA applied to excitation spectra of lac dye (dark-blue), cochineal (green) and brazilwood (red). The data of the Ajuda Songbook is predicted within the model (*marked in black with an arrow*).

A2.2. Purple organic colorants

The purple color is found in both the Lineage Book and in the Theology Treatise. In the first, the purple is embellishing every decorated initial, with filigree motifs. In the second it appears in a priveliged place, as a writing vehicle and to complete the illuminations, **Figure A2.3**.

The combined use of FORS and microspectrofluorimetry was essential for the characterization of the purple color in both case studies. Apparent absorption (−log*R*), together with fluorescence emission and excitation spectra, correlate well with the data obtained for a orcein-dyed silk reference (*Lasallia pustulata*[86]). The Lineage Book purple, characterized by absorption maxima between 575 and 578 nm with a shoulder at 540–545 nm, is similar to the dyed silk reference, with absorption maxima at 580 nm and a shoulder at 540 nm, see **Figure A2.4** and **Table A2.2**. Likewise, the purple paints absorption matches well the excitation spectrum, which is well resolved. On the other hand, the emission spectrum is characterized by a broad band with maxima between 590 and 600 nm, which compares well with the 602 nm value for the dyed silk reference. The identification of orcein by Raman spectroscopy in situ was not possible due to the high fluorescence observed. For the purple in the Theology Treatise, the absorption maxima at 570 nm, and the excitation maxima at 580 nm with a shoulder at 535 nm correlate with the data acqured from the Lineage Book, and the reference. The same can be said about the emission maximum at 600 nm, which is a match with the orcein reference see Image and **Table A2.2**.

[86] See Melo MJ, Nabais P, Guimarães M, Araújo R, Castro R, Oliveira MC, Whitworth I. Organic dyes in illuminated manuscripts: an unique cultural and historic record. Phil Trans R Soc A. 2016;374(2082):20160050, for the experimental condition and more information about the purples identified.

Figure A2.3. The purple color was applied as a filigree in the capitulars of the Lineage Book (*top*) and in the Theology Treatise (*bottom*).

However, although the excitation and emission signal are similar, the infrared spectra indicate the use of different binders. For the Lineage Book a protein binder was used, while for the Theology Treatise a polysaccharide was chosen, see **Figure A2.4**. The use of orcein was extensive after the 14th c. onwards, so its finding in the Lineage Book and the Theology Treatise is in accordance with the literature, however, one cannot set aside another purple, *folium*, since its fluorescence spectra are very coincidental to that of orcein. Future studies on these purples are ongoing, encompassing the creation of a reference database as extensive and thorough as that build for red lake pigments.

Table A2.2. Absorption (−log*R*), excitation and emission maxima for the purple from the Lineage Book and Theology Treatise, compared with a natural orcein reference.

	λabs (nm)	λexc (nm)	λem (nm)
Lineage Book, purple	575-578 540-545 (*sh*)	571-583 535-549 (*sh*)	590-600
Theology Treatise	570	580 535 (*sh*)	600
Natural orcein reference[2]	580 540 (*sh*)	574 550 (*sh*)	602

2 – Silk dyed with *Lasallia pustulata* lichens.

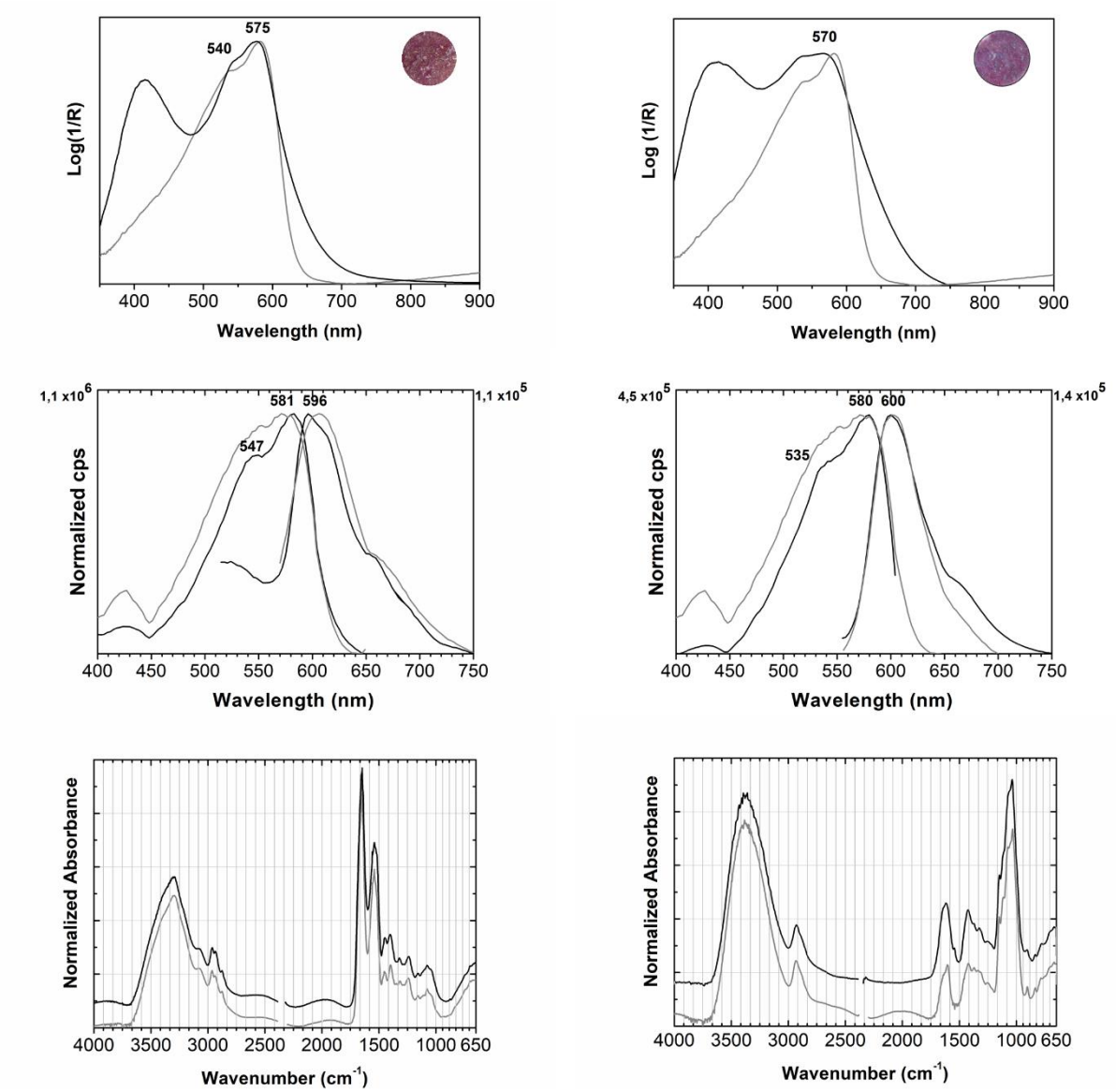


Figure A2.4. Purple in Lineage Book, page 12 (*left*) and in the Theology Treatise fol. 43r (*right*). Spectra for natural orcein reference (*grey*, silk dyed with *Lasallia pustulata* lichens) and for the purple color (*black*): (*top*) apparent absorption (-log*R*); (*middle*) excitation and emission spectra; (*bottom*) infrared showing the presence of a proteinaceous binder (*grey*).

Appendix 3. The Ajuda Songbook

A3.1. Analytical techniques

The main analyses of the color paints in the Ajuda Songbook were performed in fols. 4, 10, 16, 17, 21, 33, 37, 40v, 43, 45, 59, 68v, 70, 75, 77, 79 and 87. While the analysis of the Lineage Book was conducted on pages 1, 12, 13, 31, 41, 44, 45 and 55.

Micro-sampling

Micro-sampling of the manuscripts was performed with a microchisel from Ted Pella microtools under a Leica KL 1500 LCD microscope, (7.1x to 115x objective) and a Leica Digilux digital camera, with external illumination via optical fibers. Micro-samples were taken under a microscope, typically of 20–50 μm in diameter and as such invisible to the naked eye; as we have not yet obtained their weight, even though micro-scales have been used, we can use its detection limit to conclude that they weigh less than 0.1 μg.

Energy dispersive X-ray fluorescence (microEDXRF)

MicroEDXRF results were obtained using an ArtTAX spectrometer of Intax GmbH, with a low-power molybdenum (Mo) X-Ray tube attaining a microspot with a spatial resolution of circa 70 μm, an X-flash detector refrigerated by the Peltier effect (Sidrift), sustained by a mobile arm (providing a major freedom in choosing the spot of analysis). The accuracy of the incident beam position on the sample is achieved through three beams crossing diodes controlled by an integrated CCD camera; the characteristic X-rays emitted by the sample (at 40º) are detected by a silicon drift electro-thermally cooled detector with a resolution of 160eV at Mn-Kα. This apparatus allows for a simultaneous multi-element analysis in the element range from Mg (magnesium, atomic number 12) to U (uranium, atomic number 92). The experimental parameters used were: 40kV of voltage, 300μA of intensity, for 120s, under Helium gas flux. Si, Mn, Cu and Pb standards were used as calibration standards in the beginning and at the end of each day of data acquisition. Three points per area for each color were carefully chosen to avoid interference from the ink in the reverse folio.

Fiber optic reflectance spectroscopy

The reflectance spectra were obtained with a reflectance spectrophotometer Ocean Optics, MAYA 2000 Pro, with single beam optical fibres, equipped with a linear silicon CCD detector Hamamatsu, with a spectral range of 200-1060 nm. The light source is a halogen lamp Ocean Optics HL-2000-HP, 20 W output, with a spectral range of 360-2400 nm. The analyses were obtained with 8 ms integration time, 15 scans, 8 box width, and acquired at 45º/45º (light source/acquisition), with a spatial resolution of 2 mm. To calibrate the equipment a white reference was used, Spectralon® standard. The spectra were acquired in reflectance mode and presented as apparent absorbance A'=log10(1/R).

Microspectrofluorimetry

Fluorescence excitation and emission spectra were recorded with a Jobin–Yvon/Horiba SPEX Fluorog 3-2.2 spectrofluorometer hyphenated to an Olympus BX51M confocal microscope, with spatial resolution controlled by a multiple-pinhole turret, corresponding to a minimum 2 µm and maximum 60 µm spot, with 50 × objectives. Beam-splitting is obtained with standard dichroic filters mounted at 45°; they are located in a two-place filter holder. For a dichroic filter of 570 nm, excitation may be carried out until about 560 nm and emission collected after about 580 nm ("excite bellow, collect above"). The optimization of the signal was performed daily for all pinhole apertures through mirror alignment, following the manufacturer's instructions, using a rhodamine standard (or other adequate reference). filters of 540 and 620 nm were used where the emission spectra were acquired exciting at 530 nm, and excitation spectra were recorded collecting the signal at 630 nm. This enables both the emission and excitation spectra to be collected with the same filter holder. A continuous 450 W xenon lamp, providing an intense broad spectrum from the UV to near-IR, is directed into a double-grating monochromator, and spectra are collected after focusing on the sample (eye view) followed by signal intensity optimization (detector reading). The pinhole aperture that controls the area of analysis is selected based on the signal-to-noise ratio. For weak to medium emitters, it is set to 8 µm, in this work for very weak signals 30 µm spot was also used (pinholes 5 and 8, respectively) with the following slits set: emission slits = 3/3/3 mm and excitation slits = 5/3/0.8 mm. Emission and excitation spectra were acquired on the same spot whenever possible.

Fourier transform infrared microspectroscopy (microFTIR)

Infrared analyses were performed using a Nicolet Nexus spectrophotometer coupled to a Continuµm microscope (15x objective) with a MCT-A detector cooled by liquid nitrogen. The spectra were collected in transmission mode, in 50 µm areas resolution setting 4 or 8 cm^{-1} and 128 scans, using a Thermo diamond anvil compression cell. For some infrared spectra the system was purged with nitrogen prior to the data acquisition; for all infrared spectra the CO_2 absorption at circa 2400-2300 cm^{-1} was removed from the acquired spectra (4000 - 650 cm^{-1}). To improve result robustness, more than one spectrum was acquired from different sample spots.

Raman microspectroscopy

Raman microscopy was carried out using a Labram 300 Jobin Yvon spectrometer, equipped with a diode laser with an excitation wavelength of 785nm (100mW), a HeNe laser operating at 632,8nm (17mW) and a solid-state laser operating at 532 nm (50mW). Spectra were recorded as an extended scan. The laser beam was focused with 50× and 100× Olympus objective lens. The laser power at the surface of the samples was varied with the aid of a set of neutral density filters (optical densities 0.3, 0.6 and 1). The laser power at the surface of the samples was between 9.5 and 0.17 mW. No evidence of ink or paint degradation was observed during spectra acquisition. More than three spectra were collected from the same sample. A silicon reference was used to calibrate the instrument.

A3.2. The materials of color in the Ajuda Songbook and the Lineage Book

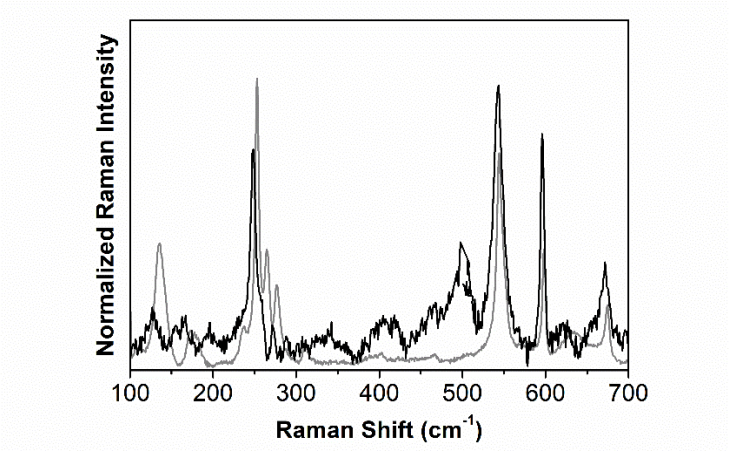


Figure A3.1. Raman spectra of a dark blue, fol. 40v. Indigo is used in clothing for shading, as proved by the molecular spectra. Raman spectra for indigo reference (*grey*) and a dark blue, fol. 40v (*black*), λ=532nm.

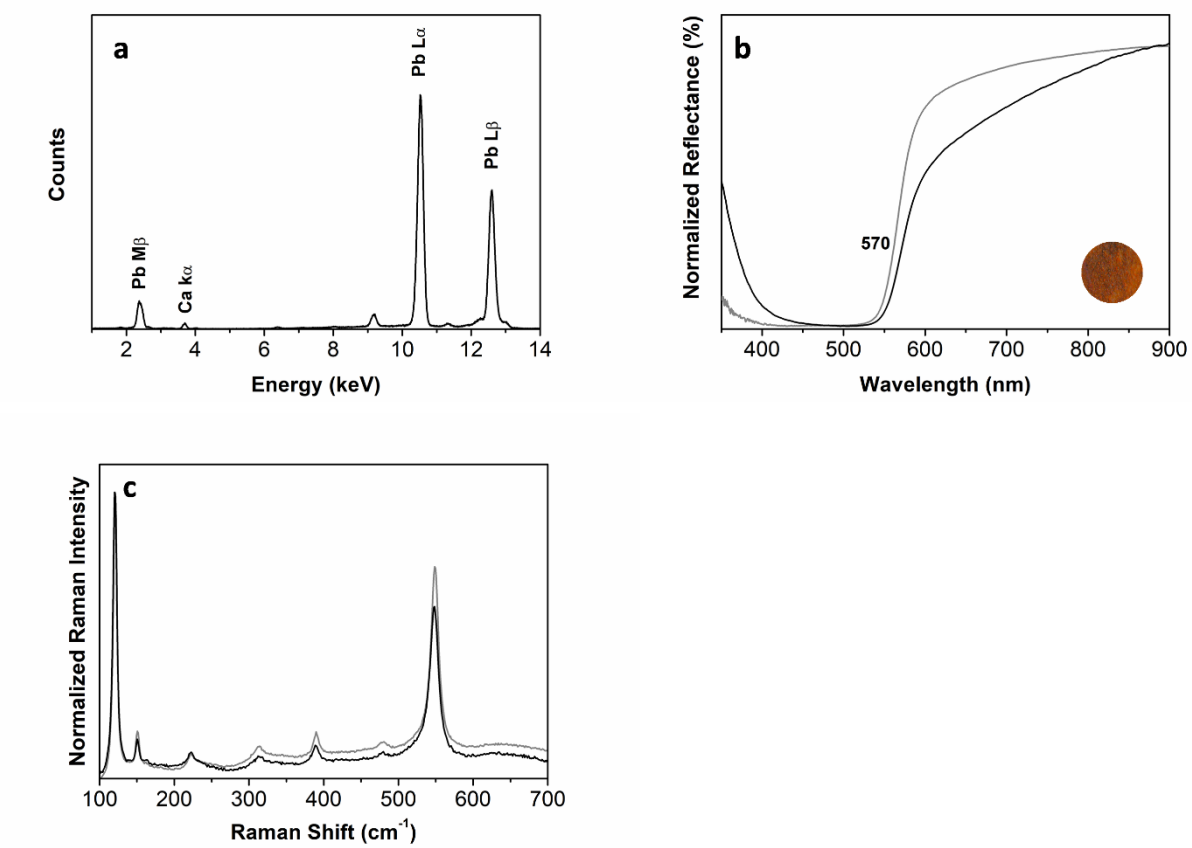


Figure A3.2. Red lead was applied in the vestments of the nobles, as proved by the molecular spectra. Spectra for red lead reference (*grey*) and an orange vestment, fol. 17 (*black*): (*a*) μ-EDXRF; (*b*) FORS; (*c*) Raman, λ=632nm.

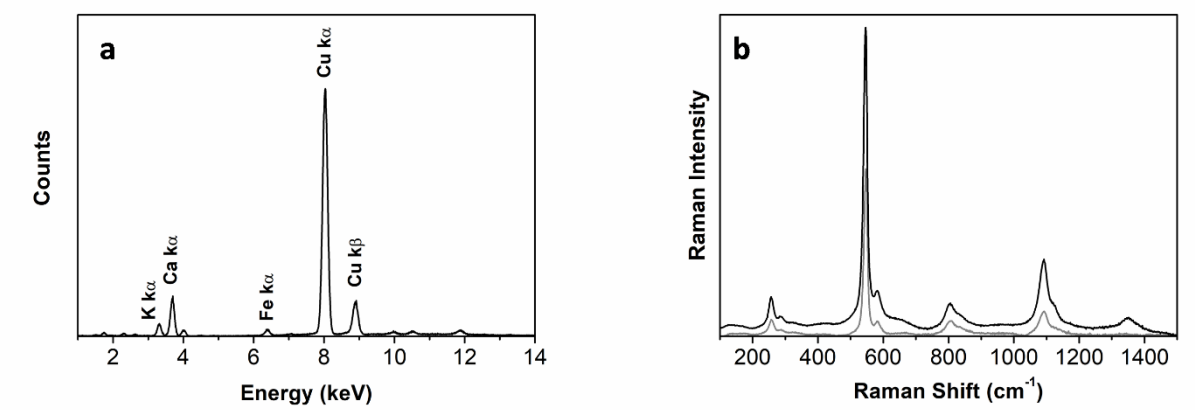


Figure A3.3. Lapis lazuli and azurite are used in the capitulars, as proved by the molecular spectra. Spectra for lapis lazuli reference (*grey*) and a blue in a capitular, fol. 21 (*black*): (*a*) μ-EDXRF; (*b*) Raman, λ=632nm.

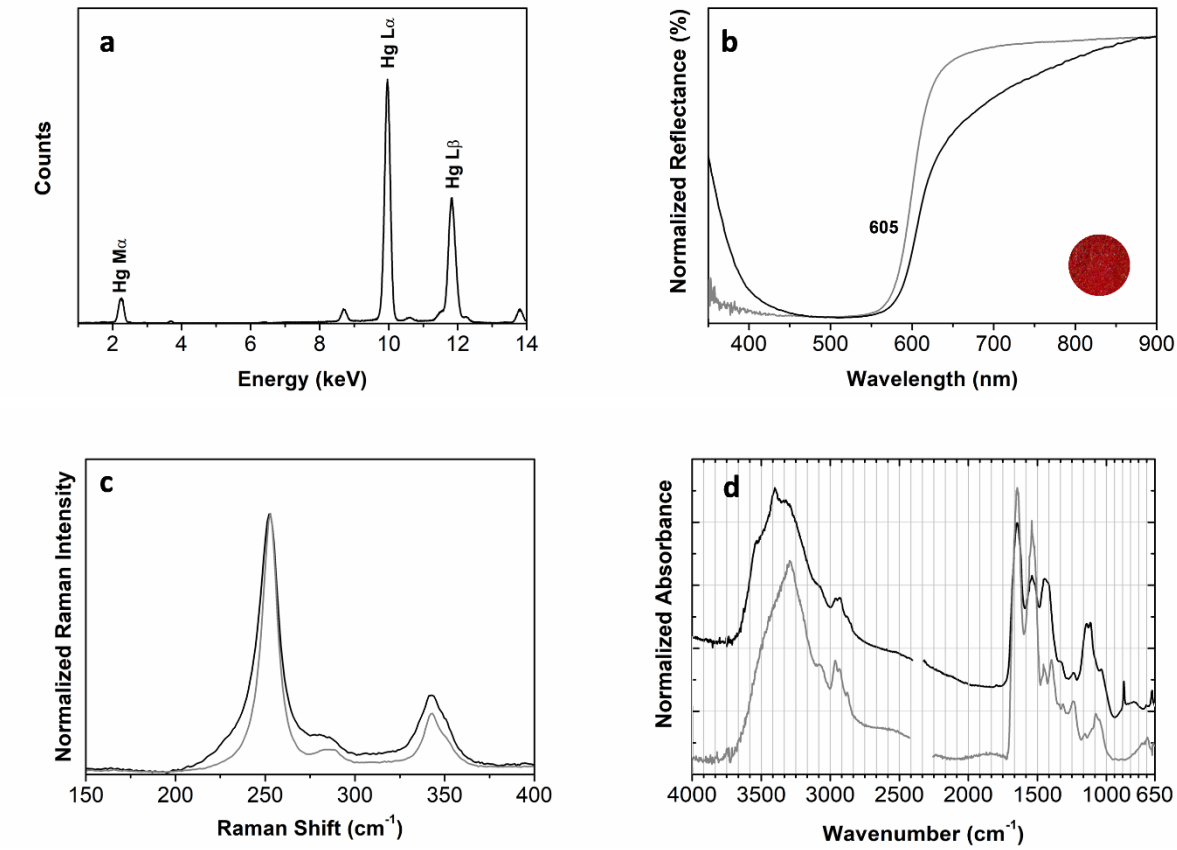


Figure A3.4. Vermilion is used in the vestments, as proved by its molecular spectra. Spectra for a vermilion reference (*grey*) and the red in the noble's vestment, fol. 40v (*black*): (*a*) μ-EDXRF; (*b*) FORS; (*c*) Raman, λ=632nm; (*d*) infrared reference spectra of protein (*grey*) and red (*black*). Vermilion does not absorb in the mid infrared region, but it causes extensive scattering, noticeable by a slope in the baseline; however, one may notice the presence of a proteinaceous binder used with this pigment, as well as gypsum and chalk.

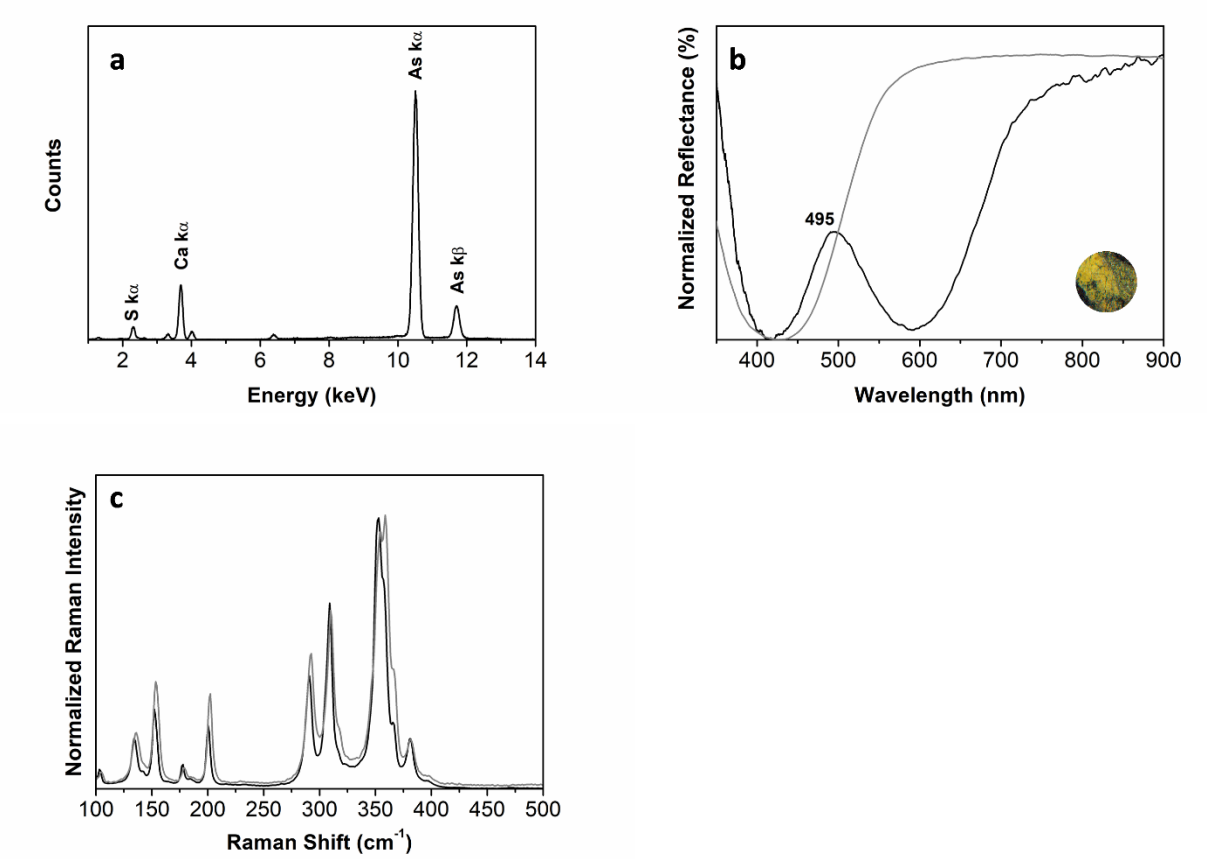


Figure A3.5. The greens are a combination of orpiment and carbon black, lapis lazuli or indigo. Spectra for an orpiment reference (*grey*) and the green in the building, fol. 4 (*black*): (*a*) micro-EDXRF; (*b*) FORS; (*c*) Raman, λ=632nm. The presence of orpiment is indicated by the As-S stretching modes fall within the 400 cm^{-1} region, while S-As-S angle deformations are expected around 200 cm^{-1}.

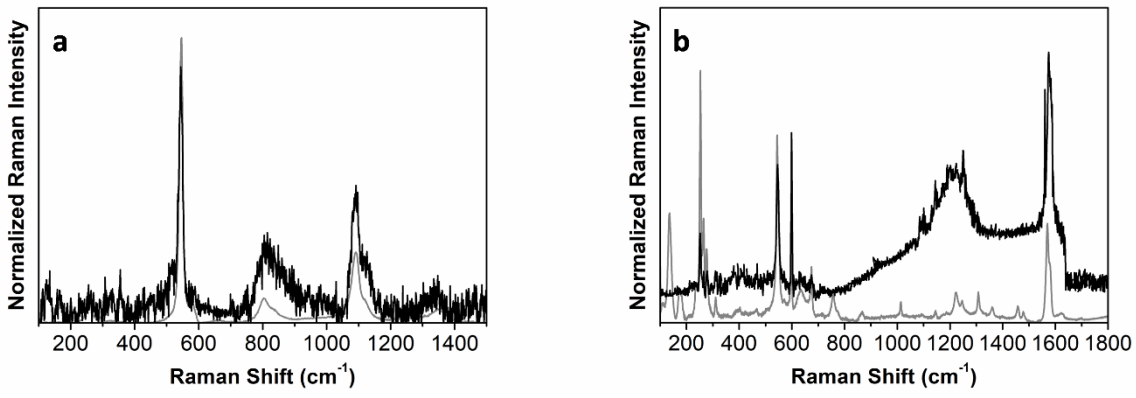


Figure A3.6. Raman spectra for greens where orpiment is mixed with (*a*) lapis lazuli or (*b*) indigo. Spectra for the lapis lazuli and indigo references (*grey*) and the greens in the building and the vestments of the dancer, fol. 17 (*black*), λ=532 & 632nm.

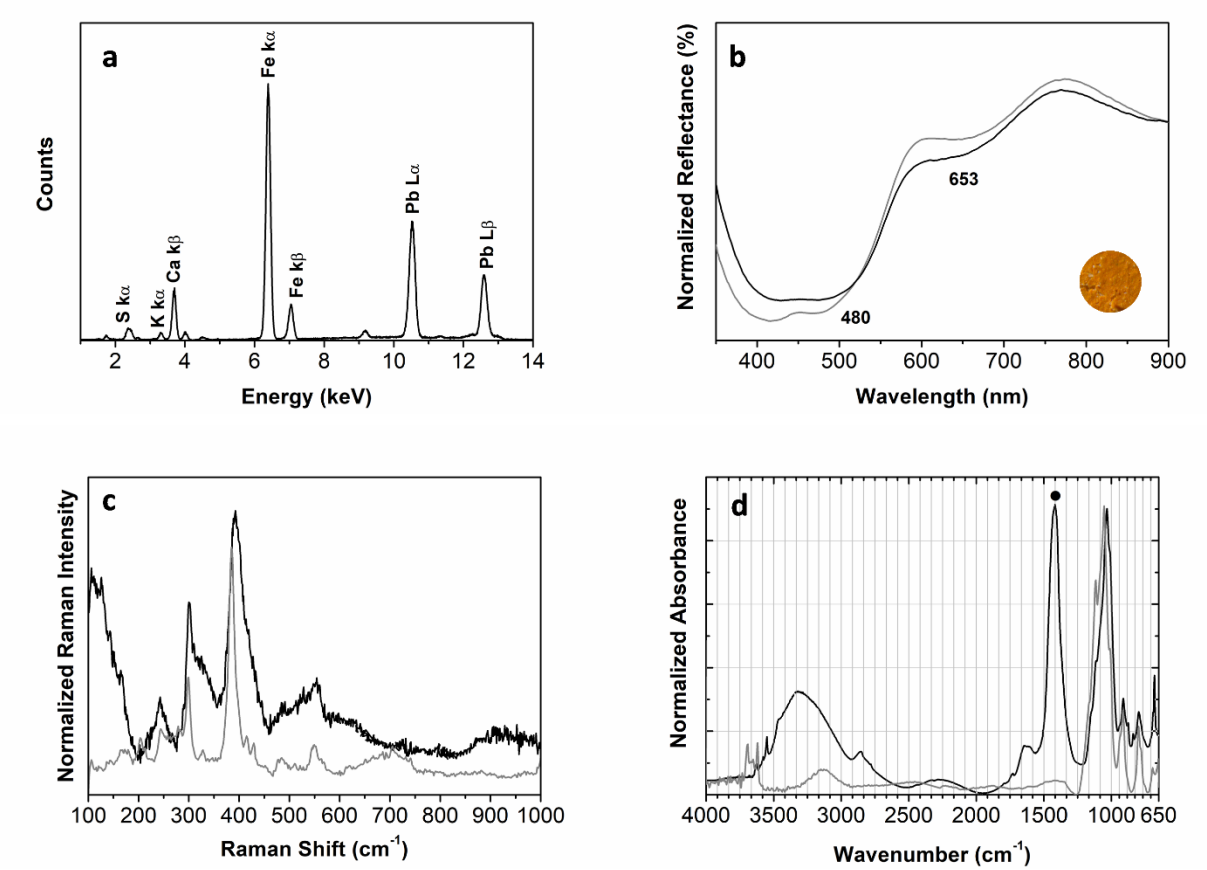


Figure A3.7. Yellow ochre is used in the architecture and musical instruments, as proved by its molecular spectra. Spectra for a yellow ochre reference (*grey*) and yellow on the building, fol. 4 (*black*): (a) µ-EDXRF; (b) FORS with the main features consistent with iron oxy-hydroxides (absorption band at 480 and 660 nm); (c) Raman, with the characteristic bands of goethite (300-392 cm^{-1}), λ=632nm; (d) infrared spectra with a band corresponding to lead white (●). The stretching vibrations of Si-O-Al and Si-O-Si around 1005-1030 cm^{-1} and in the 3600-3700 cm^{-1} are consistent with an ochre.

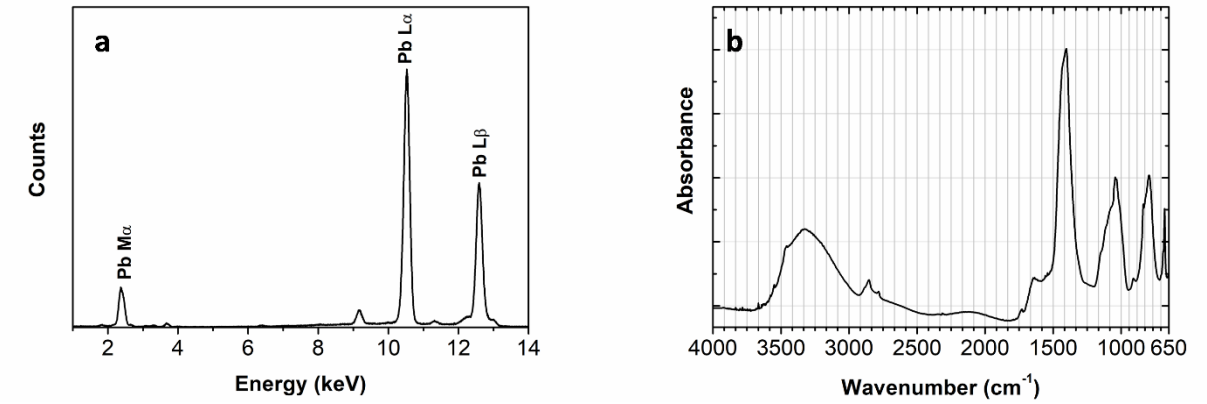


Figure A3.8. White lead is used to highlight the colors. Spectra for the white on the building, fol. 17: (*a*) µ-EDXRF; (*b*) infrared spectra, with a polysaccharide binder.

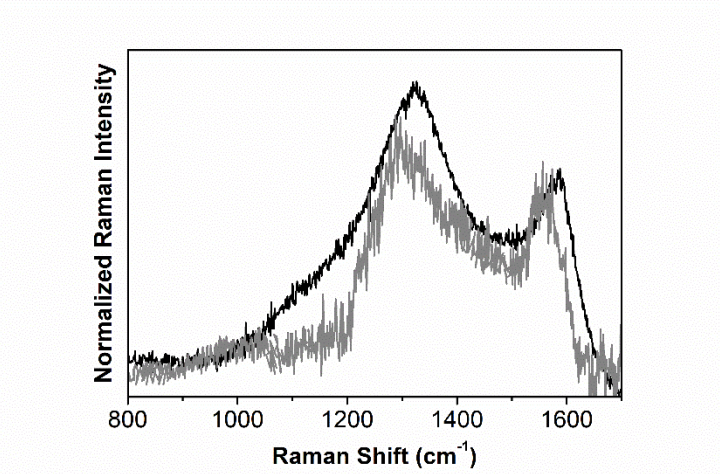


Figure A3.9. Raman spectra of carbon black reference (*grey*) and black contour from the architecture, fol. 17 (*black*), λ=632nm. Carbon black gives a weak Raman band at 1355 cm^{-1} in addition to the broad ~1580 cm^{-1}.

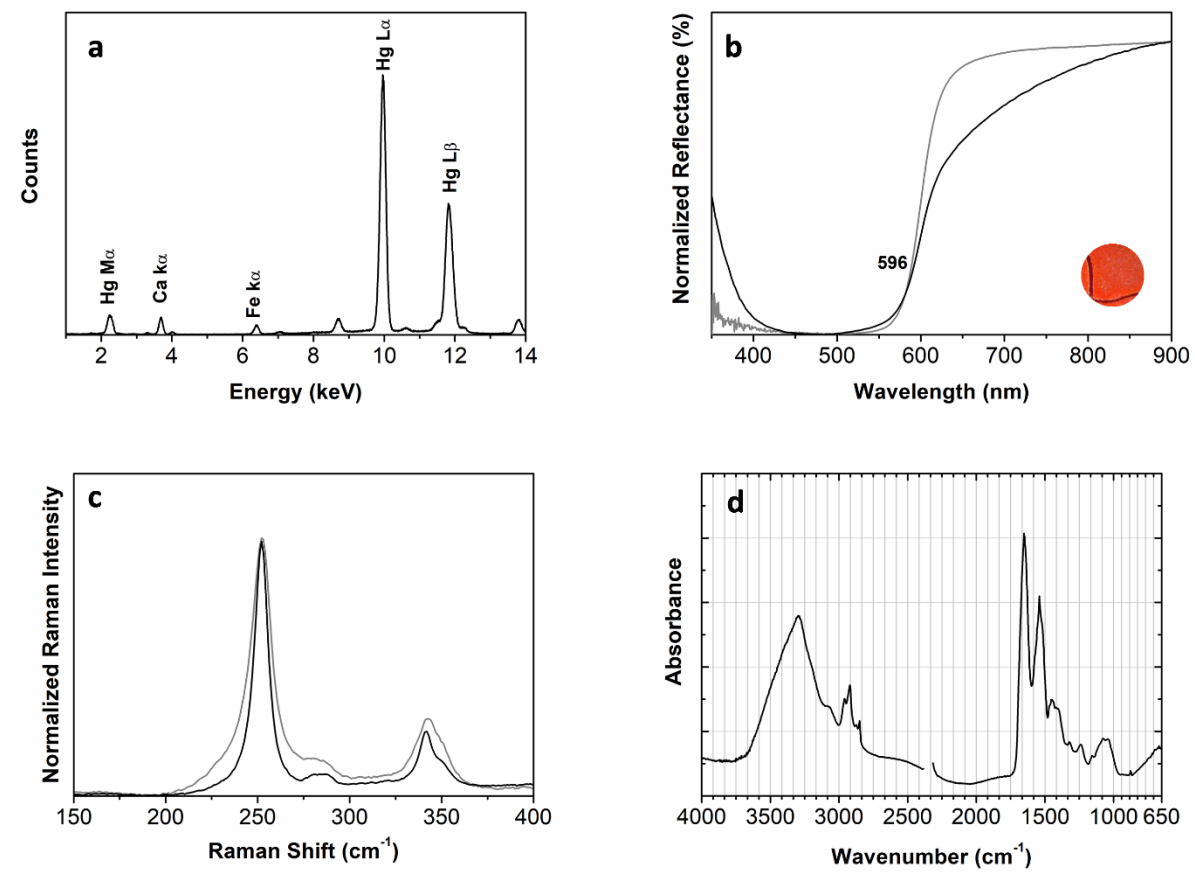


Figure A3.10. Vermilion is used in the initials of the Lineage Book, as proved by its molecular spectra. Spectra for a vermilion reference (*grey*) and a red initial, page 12 (*black*): (*a*) μ-EDXRF; (*b*) FORS; (*c*) Raman, λ=632nm; (*d*) infrared spectra with the characteristic features of a protein binder.

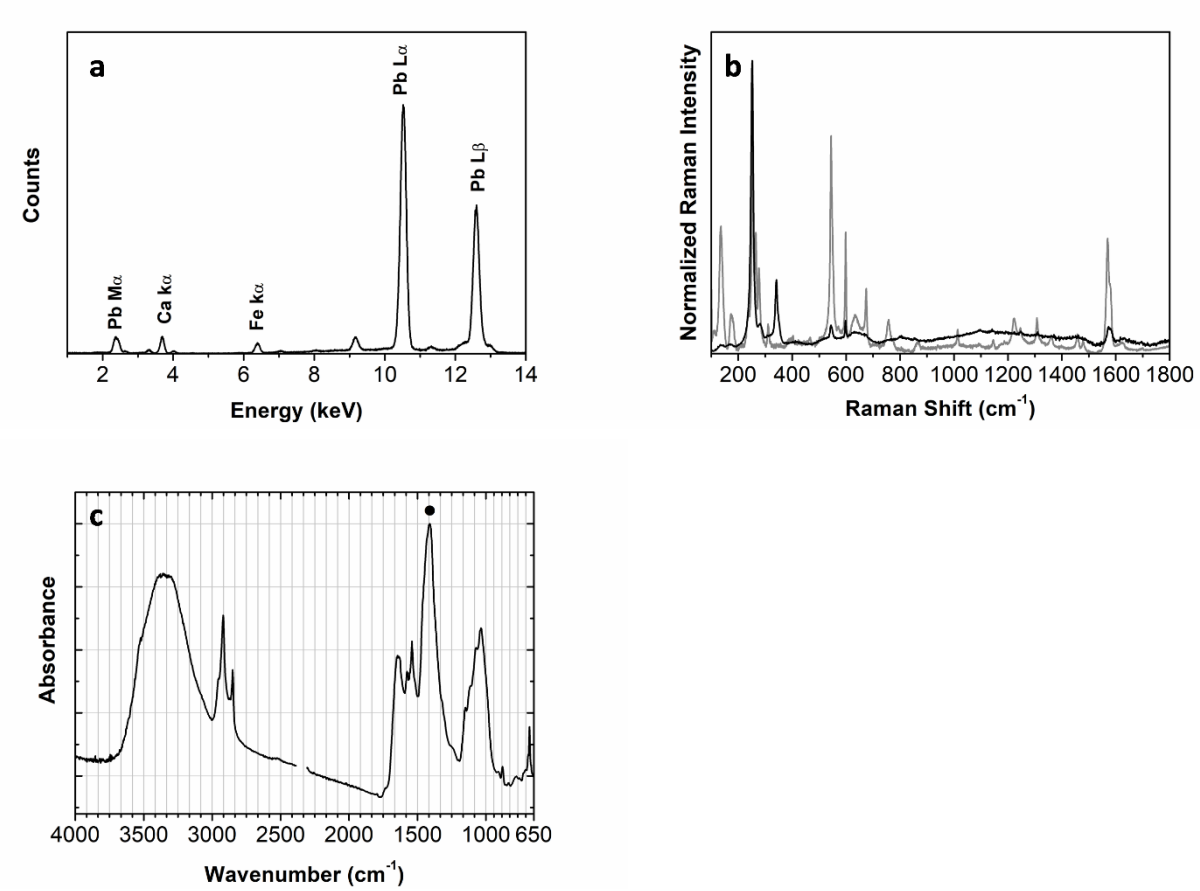


Figure A3.11. Indigo is used in the initials of the Lineage Book, as proved by its molecular spectra. Spectra for an indigo reference (*grey*) and blue in an initial, page 45 (*black*): (*a*) μ-EDXRF; (*b*) Raman, λ=632nm; (*c*) infrared spectra with a band corresponding to lead white (●).

A3.3. Folios and areas of analysis

Areas of analysis for μ-EDXRF (), FORS (), μ-sampling (), Raman in-situ () and microspectrofluorimetry in-situ ().

Figure A3.12. Ajuda Songbook, folio 4, © Ajuda Library.

Figure A3.13. Ajuda Songbook, folio 10, © Ajuda Library.

Figure A3.14. Ajuda Songbook, folio 16, © Ajuda Library.

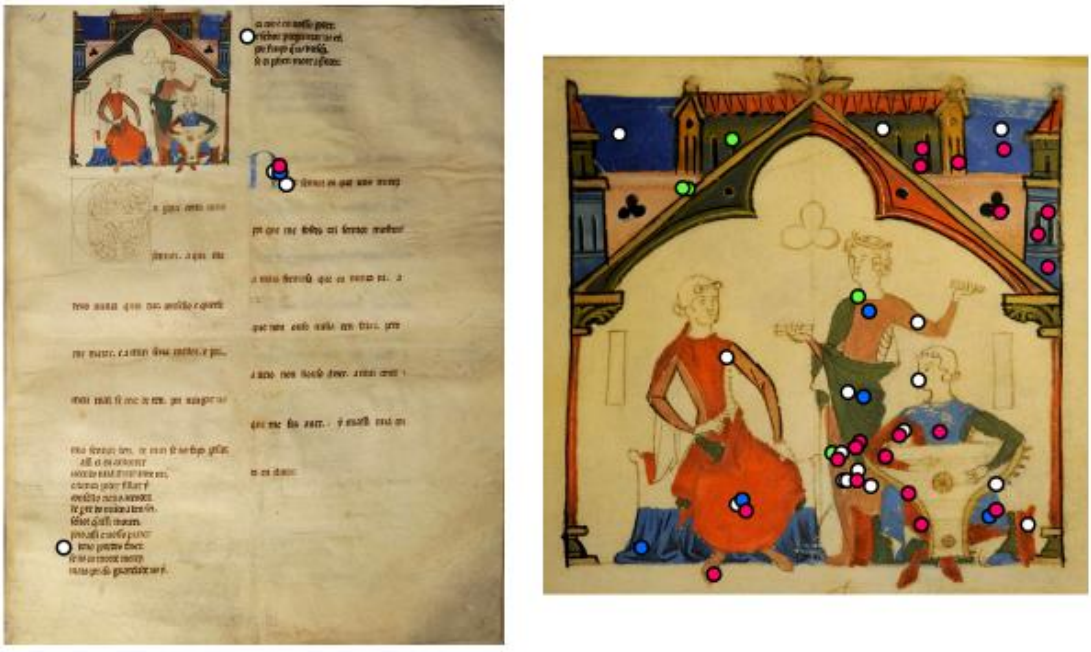


Figure A3.15. Ajuda Songbook, folio 17, © Ajuda Library.

Figure A3.16. Ajuda Songbook, folio 21, © Ajuda Library.

Figure A3.17. Ajuda Songbook, folio 33, © Ajuda Library.

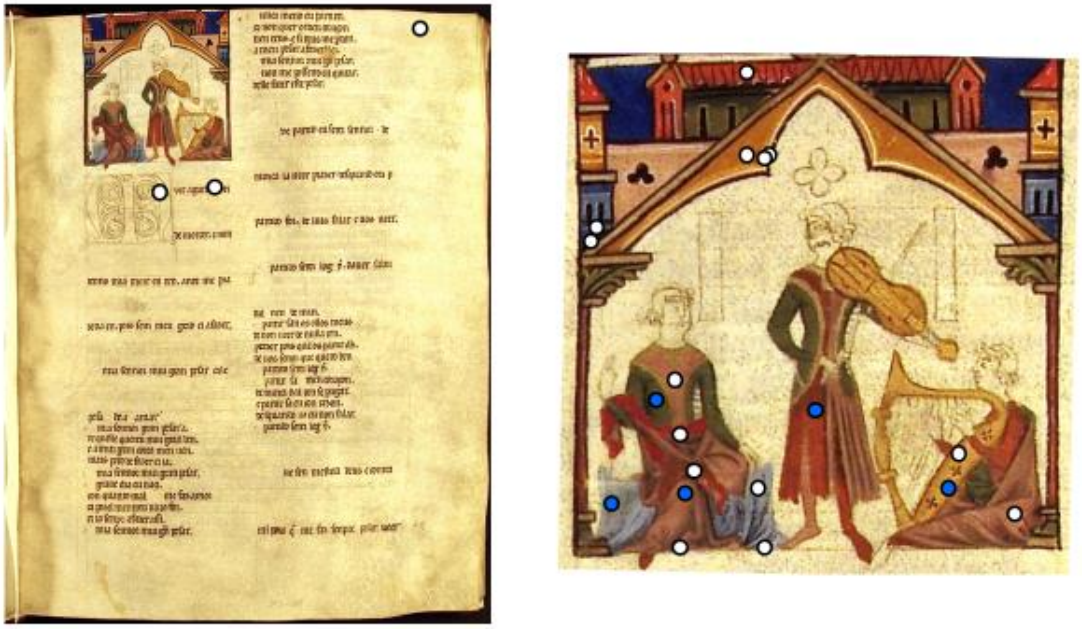


Figure A3.18. Ajuda Songbook, folio 37, © Ajuda Library.

Figure A3.19. Ajuda Songbook, folio 40v, © Ajuda Library.

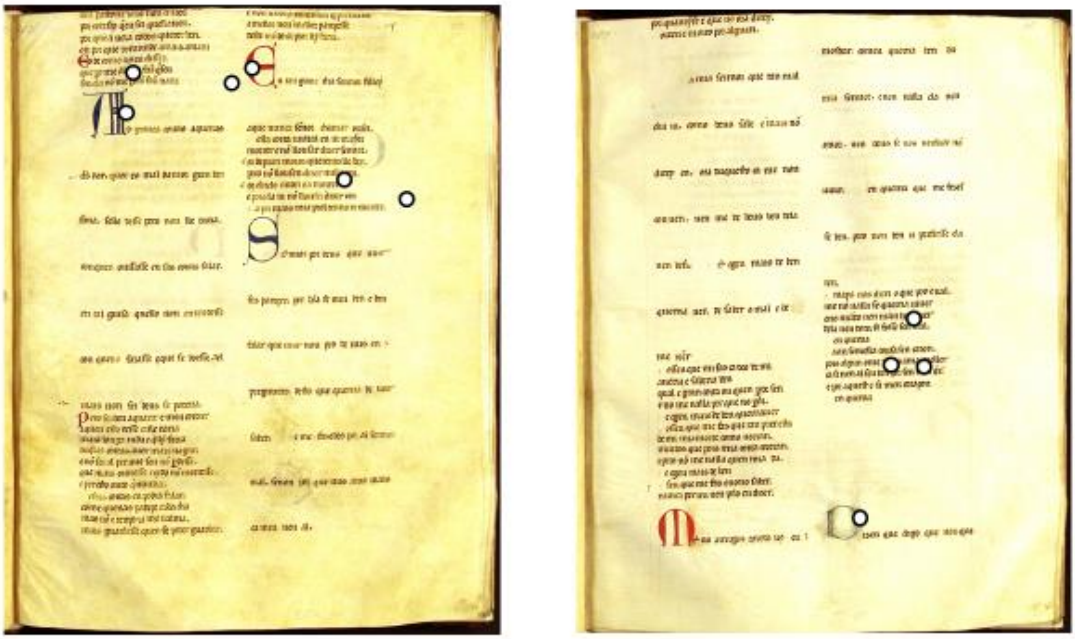


Figure A3.20. Ajuda Songbook, folios 43 (*left*) and 45 (*right*), © Ajuda Library.

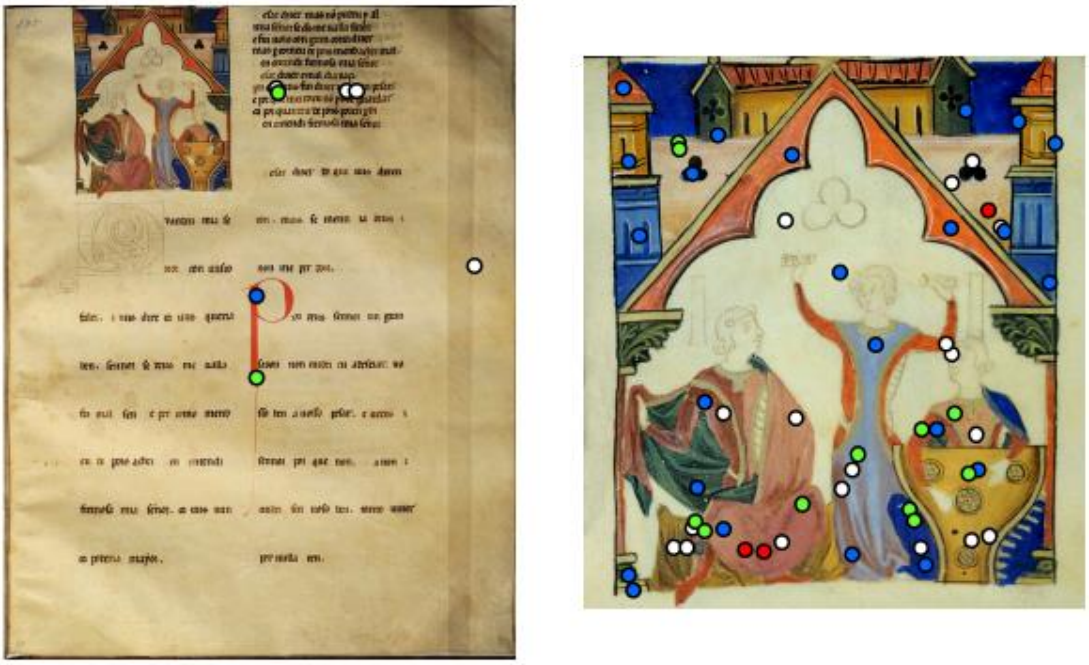


Figure A3.21. Ajuda Songbook, folio 59, © Ajuda Library.

Figure A3.22. Ajuda Songbook, folios 68v (*left*) and 70 (*right*), © Ajuda Library.

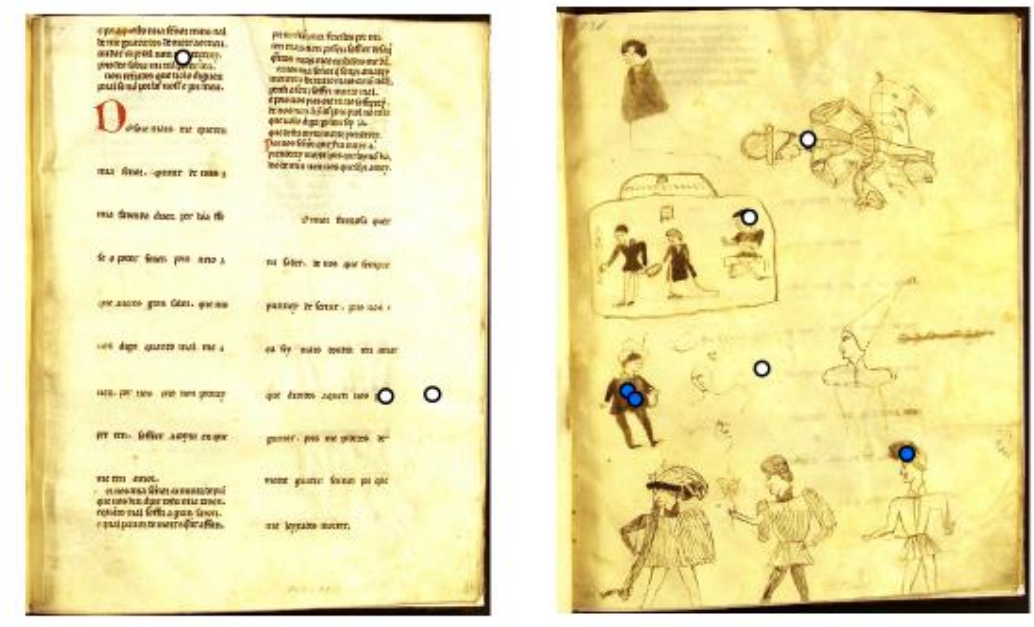


Figure A3.23. Ajuda Songbook, folios 75 (*left*) and 77 (*right*), © Ajuda Library.

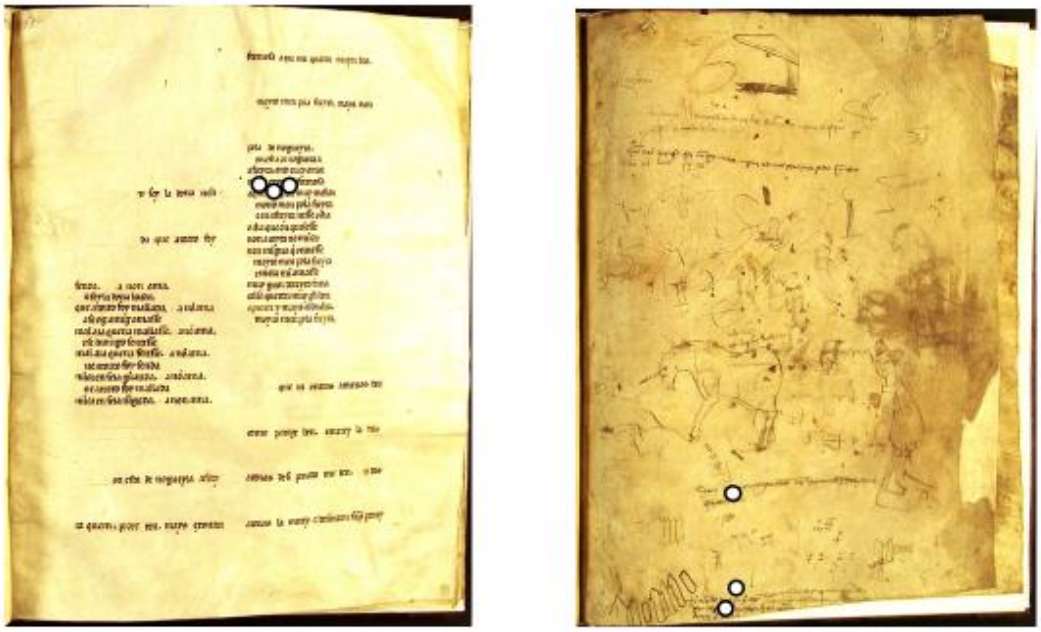


Figure A3.24. Ajuda Songbook, folios 79 (*left*) and 87 (*right*), © Ajuda Library.

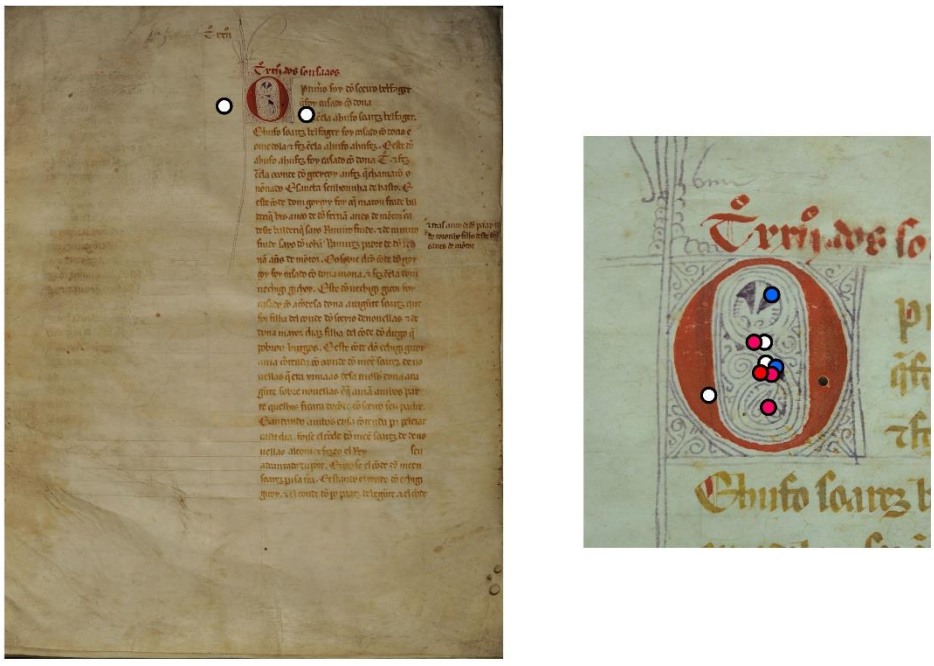


Figure A3.25. Lineage Book, page 1, © Ajuda Library.

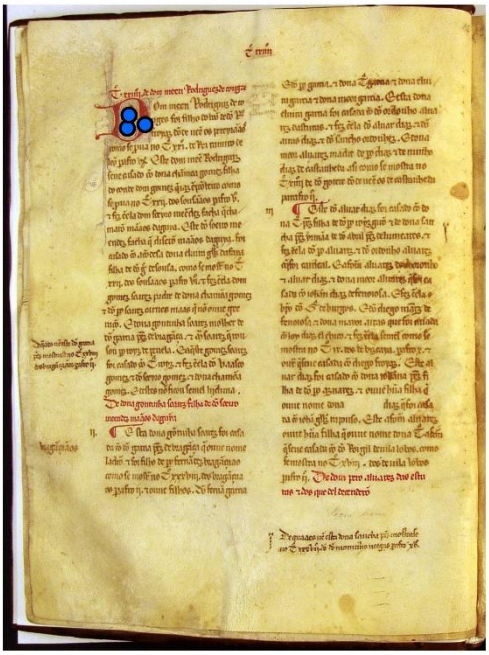


Figure A3.26. Lineage Book, page 12, © Ajuda Library.

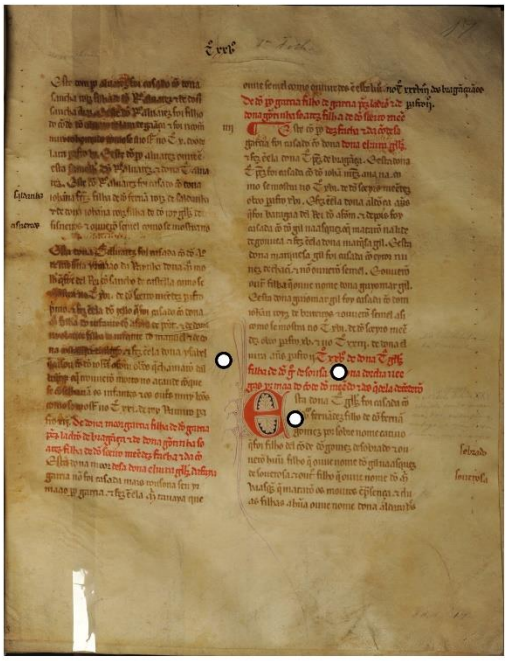


Figure A3.27. Lineage Book, page 13, © Ajuda Library.

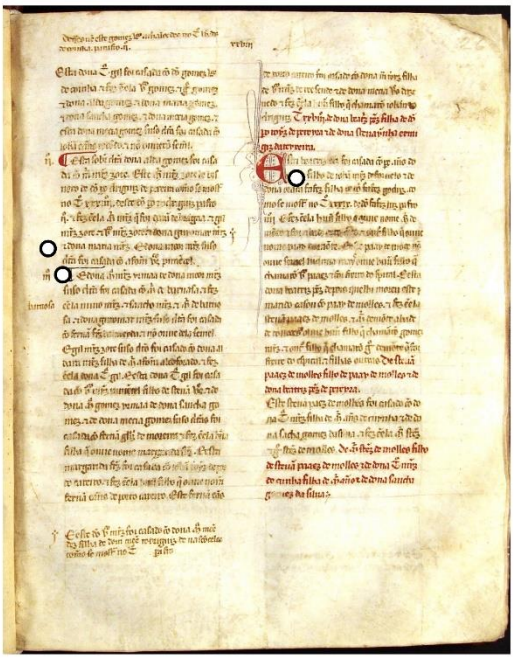


Figure A3.28. Lineage Book, page 31, © Ajuda Library.

Figure A3.29. Lineage Book, pages 41 (*left*) and 44 (*right*), © Ajuda Library.

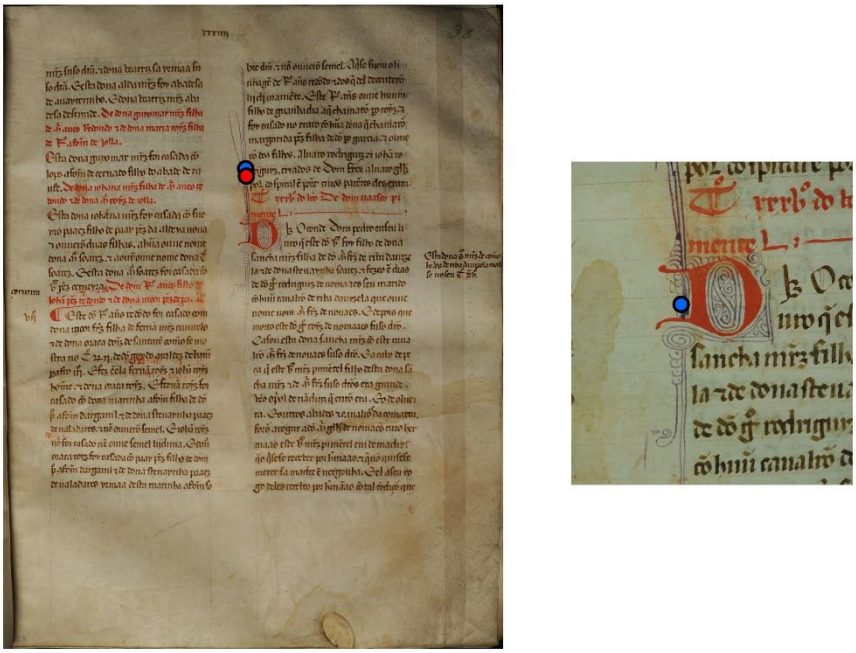
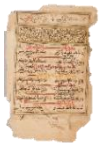


Figure A3.30. Lineage Book, page 55, © Ajuda Library.

Appendix 4. Islamic manuscripts

A4.1. The materials of color of the Islamic manuscripts

Table A4.1. Conservation condition and materials identified in the manuscripts analyzed.

	Koran		*Teology Treatise*	*Biography of the Prophet*	*Manuscript 19*	*Poemas of Al-Sarishi*
	1198		**14th c.**	**1468**	**1485**	**15th c.**
						
Suport	Parchment	Paper	Paper	Paper	Paper	Paper
Inks conservartion condition	*	*	***	***	***	***
Suport conservation condition	**	*	*	*	*	*
Colorants	    -  	    -	  -  -	    -	   -  -	    -
Contours						
Binders						

Conservation state: *very poor - ****good

Materials: organic colorants; gold; pigments with As, including pararealgar; vermilion; orpiment; lápis lazúli; azurite; copper green carbon black; proteinaceous binder polissacharide.

Table A4.2. Manuscript description, including the date and possible place of production, text block and binding description and dimensions, as well as writing and decoration characteristics.

	Koran	***Mss 19***	***Theology Treatise***	***Prophet's Biography***	***Al-Sarishi Poems***
Date	1198	1485	14th c.	1468	15th c.
Material author	Anonymous	Alfa Mahmud Kati	Anonymous	Qadi Iyad of Ceuta or by an anonymous scribe	Anonymous
Place of production	Ceuta, Spain	n.a.	n.a.	Ceuta, Spain	Jerez de la Frontera, Spain
Text block	64 parchment folios and one paper folio (fol 1), probably a later addition	250 paper folios, paginated in graphite	98 paper folios	229 paper folios	16 paper folios
Structure and binding	All folios are separated and unsewn	Existent Islamic binding	All folios are separated and unsewn	All folios are separated and unsewn	All folios are separated and unsewn
Text block dimensions (h x w)	212 x 197 mm	120 x 120 mm	240 x 175 mm	208 x 138 mm	140 x 100 mm
Writing	Black ink with annotations in blue and red	Black and red ink. Some notes and comments can be found in the margins	Black, dark red and purple	Black, gold, red and blue. Some notes and comments can be found in the margins	Black, yellow, red, dark red and blue
Decoration	Marks in gold, orange, red and blue colors, both in the text and in the margins	Geometrical and vegetal decoration in blue, orange, dark red and black	Vegetal decoration in gold and purple	Red and blue decorative panels	Decorative panels of the same colors as the writing as well as gold

Figure A4.1. Vestigial color found in the Koran, fol. 30r.

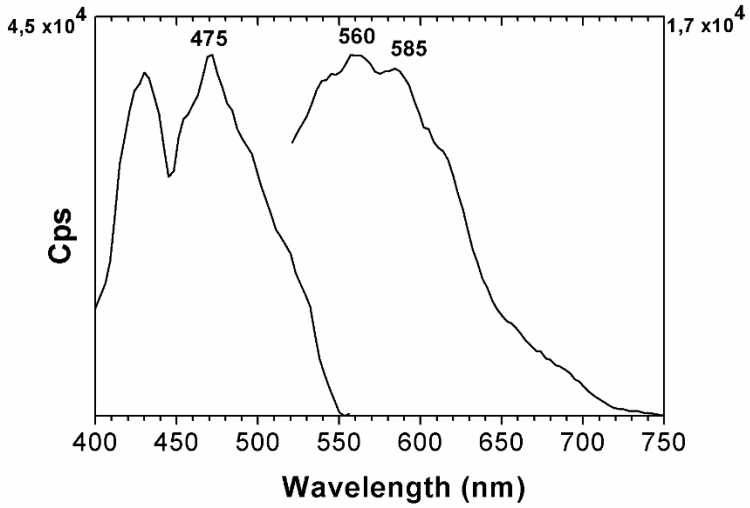


Figure A4.2. Excitation and emission spectra for the organic red present in fol.30 of the Koran.

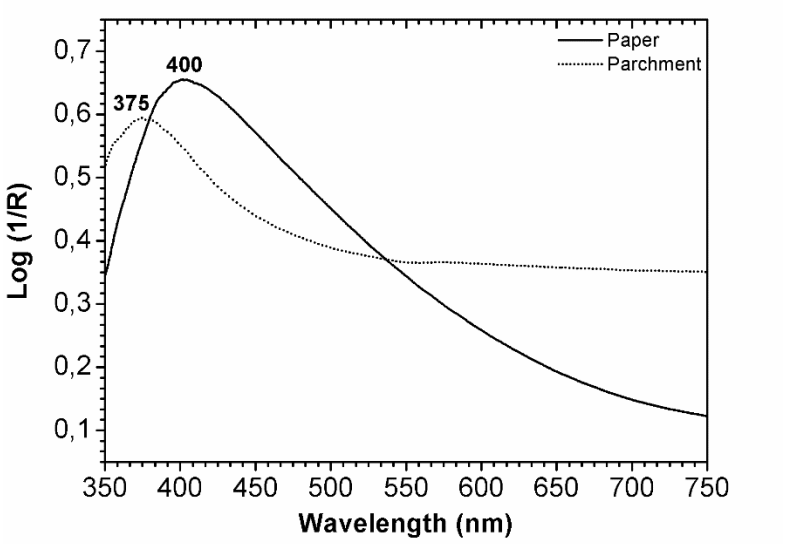


Figure A4.3. Reflectance spectra of parchment and paper.

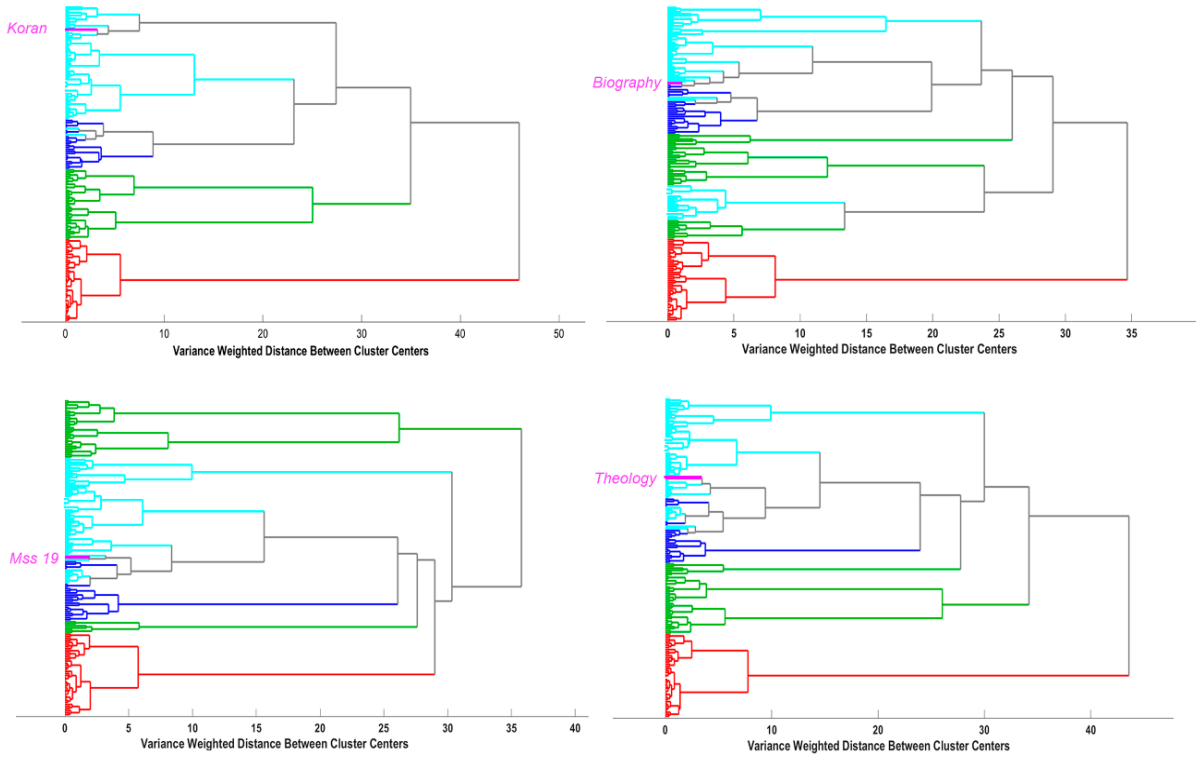


Figure A4.4. Dendrogram generated by HCA applied to excitation spectra using a model composed of data acquired from historically accurate reconstructions (lac dye (*blue*), kermes (*dark-blue*), cochineal (*green*) and brazilwood (*red*)). The data from the Islamic manuscripts was predicted within the model (*pink*).

A4.2. Folios and areas of analysis

Areas of analysis for μ-EDXRF (), FORS (), μ-sampling (), Raman in-situ () and microspectrofluorimetry in-situ ().

KORAN

Figure A4.5. Koran, folio 1, © *Fondo Ka'ti.*

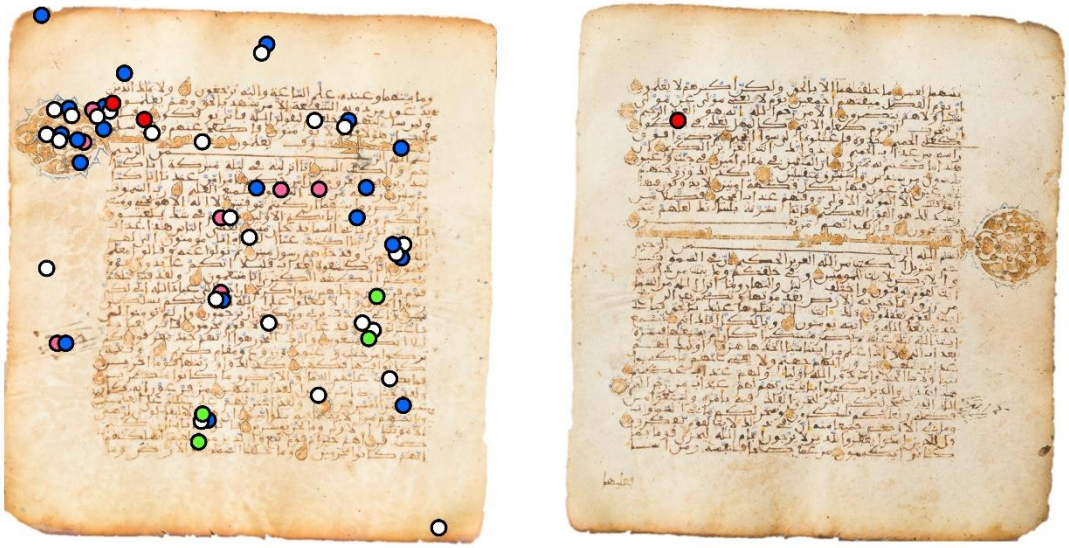


Figure A4.6. Koran, folio 30, © *Fondo Ka'ti.*

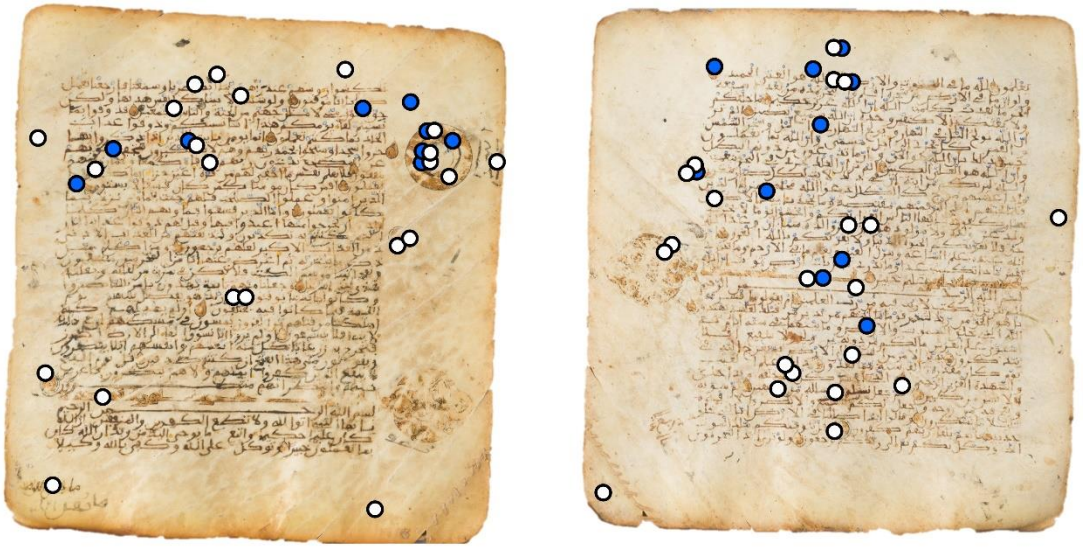


Figure A4.7. Koran, folio 47, © *Fondo Ka'ti.*

Figure A4.8. Koran, folio 70, © *Fondo Ka'ti.*

THEOLOGY TREATISE

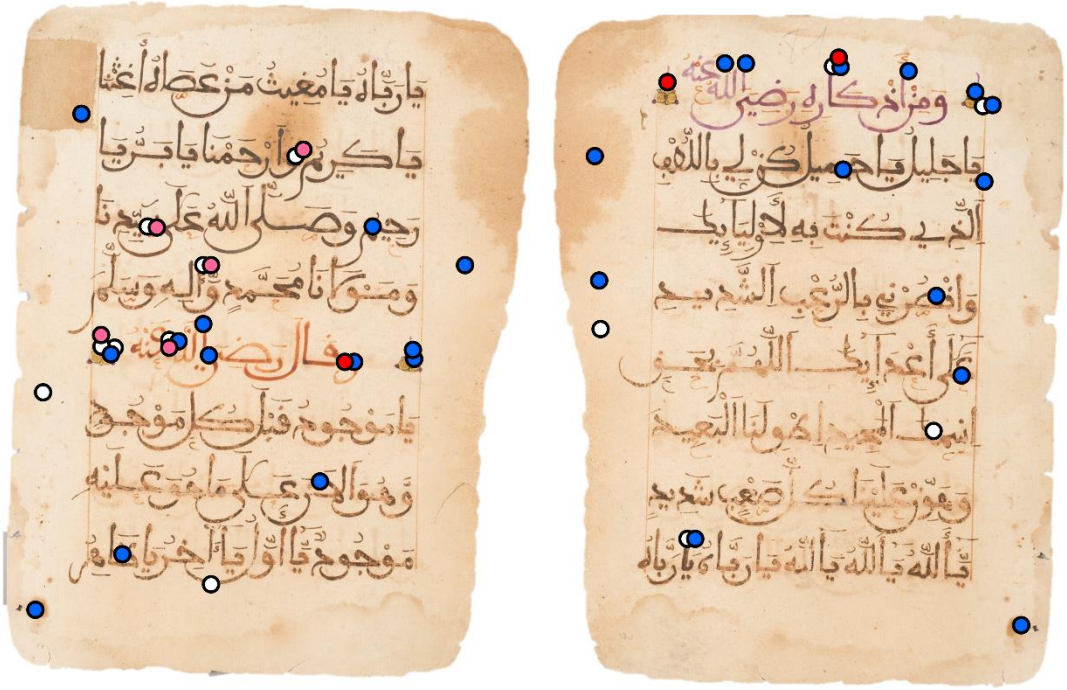


Figure A4.9. Theology Treatise, folio 17, © *Fondo Ka'ti.*

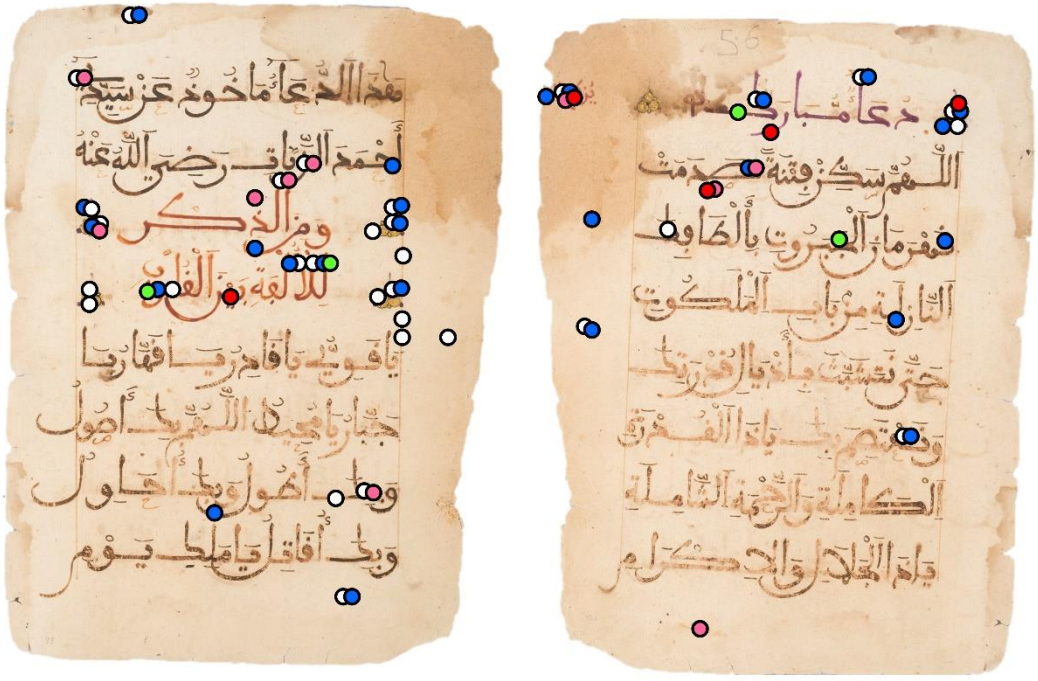


Figure A4.10. Theology Treatise, folio 43, © *Fondo Ka'ti.*

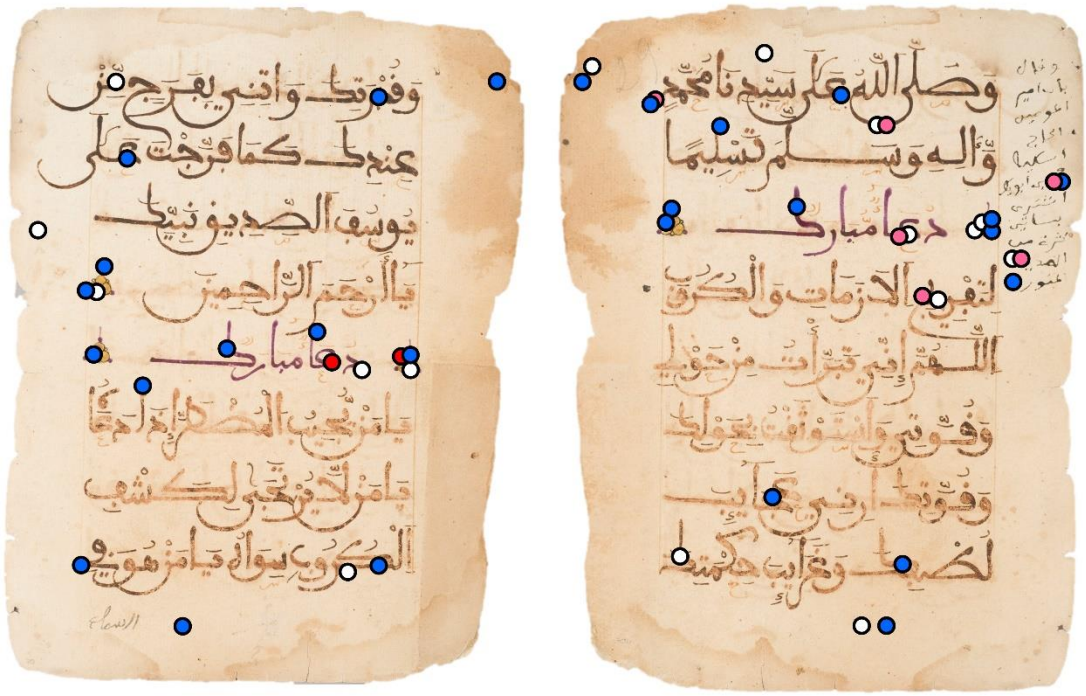


Figure A4.11. Theology Treatise, folio 70, © *Fondo Ka'ti.*

BIOGRAPHY OF THE PROPHET

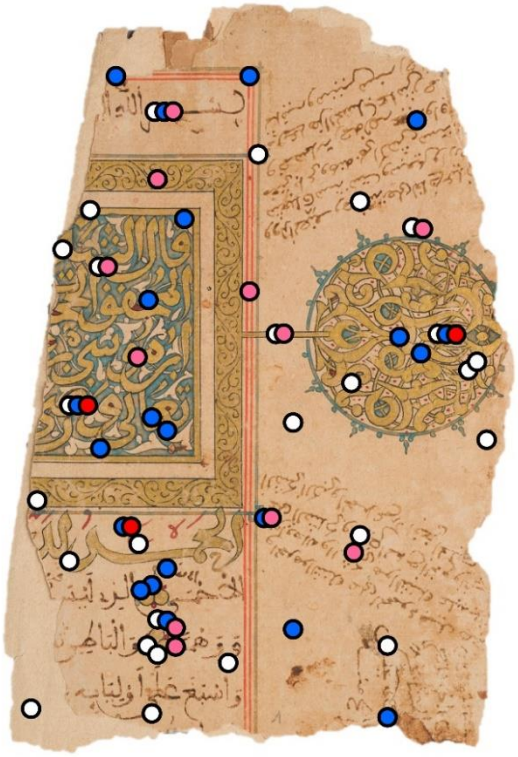


Figure A4.12. Biography of the Prophet, folio 1, © *Fondo Ka'ti.*

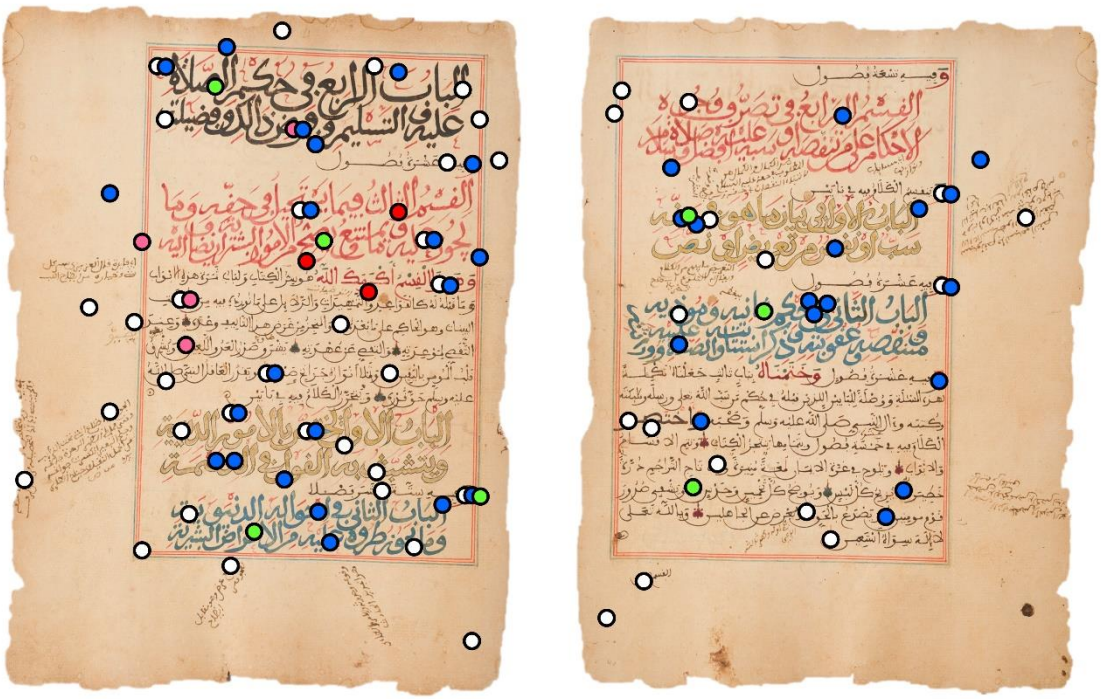


Figure A4.13. Biography of the Prophet, folio 4, © *Fondo Ka'ti.*

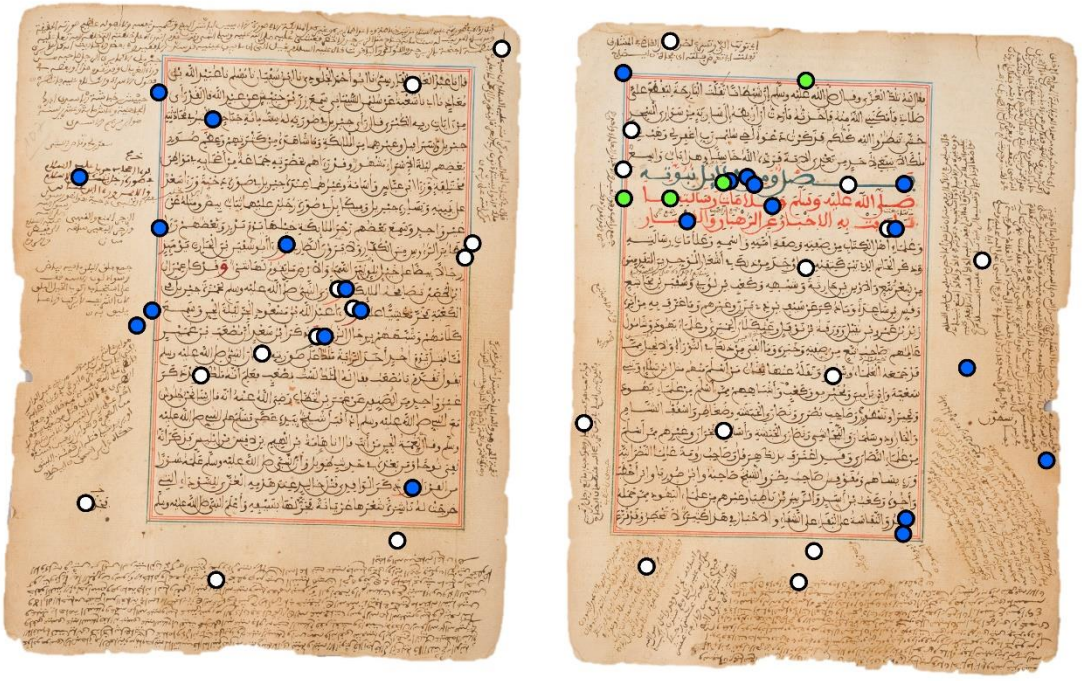


Figure A4.14. Biography of the Prophet, folio 107, © *Fondo Ka'ti.*

MANUSCRIPT 19

Figure A4.15. Manuscript 19, folio 19v and 18r, © *Fondo Ka'ti.*

Figure A4.16. Manuscript 19, folio 18v and 19r, © *Fondo Ka'ti.*

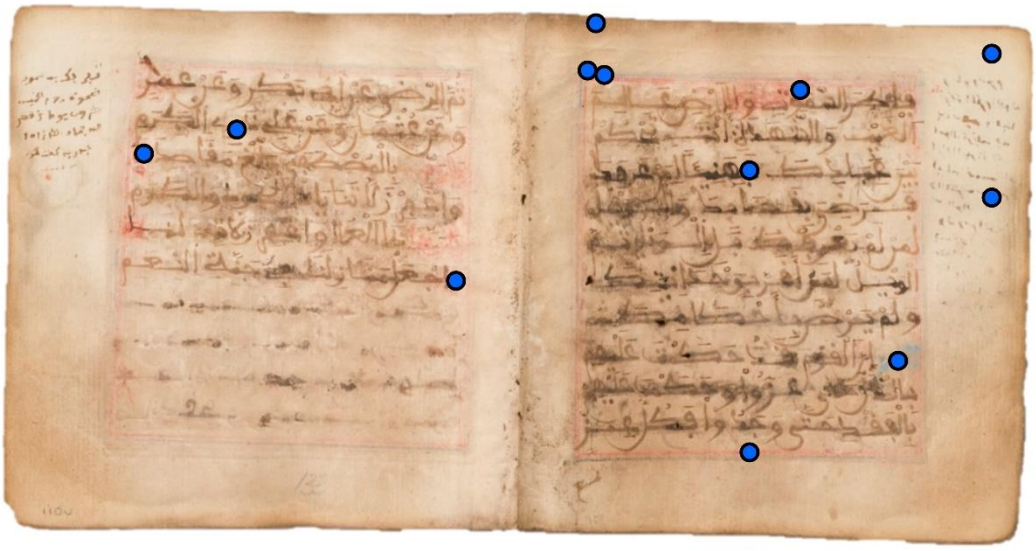


Figure A4.17. Manuscript 19, folio 19v and 18r, © *Fondo Ka'ti.*

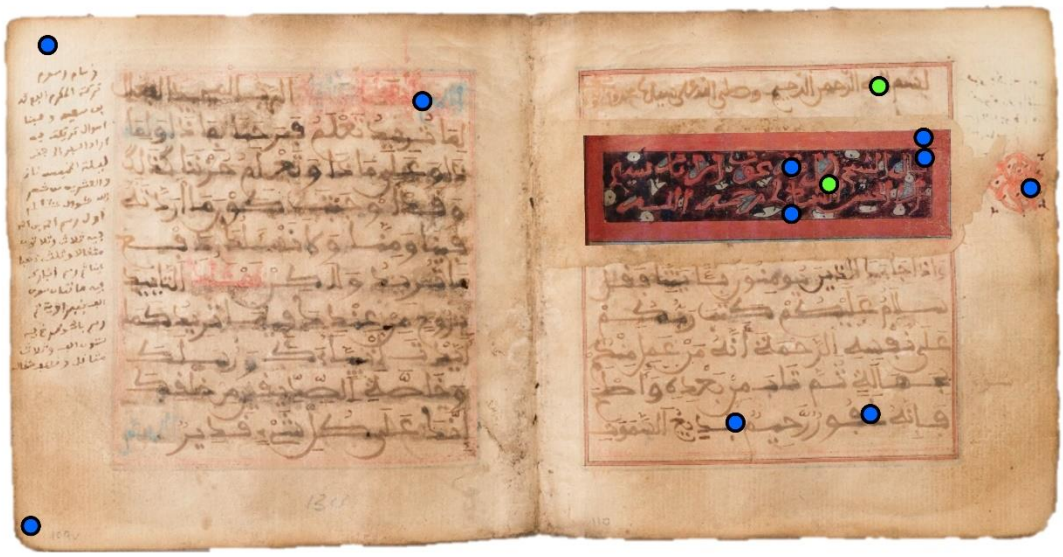


Figure A4.18. Manuscript 19, folio 109v and 110r, © *Fondo Ka'ti.*

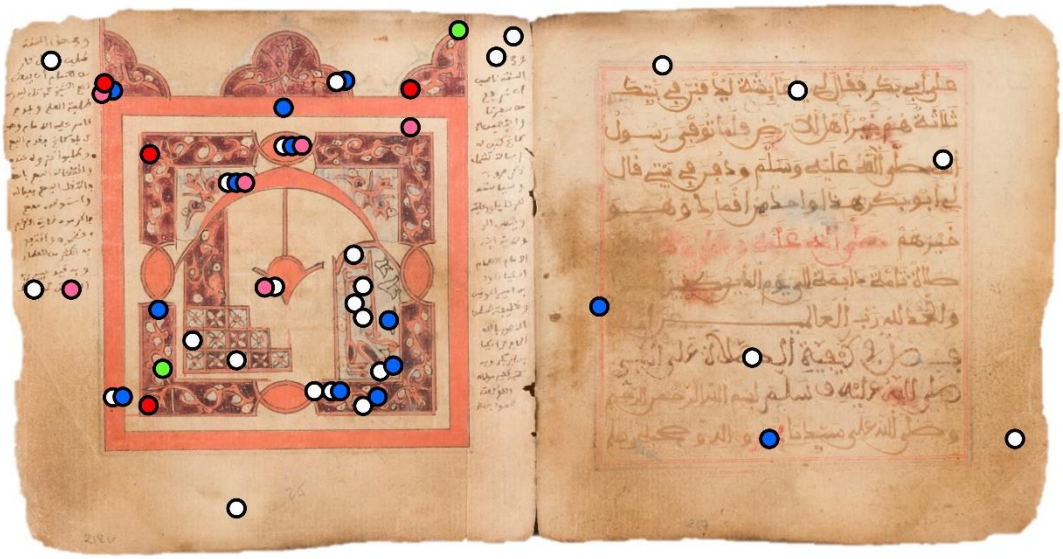


Figure A4.19. Manuscript 19, folio 218v and 217r, © *Fondo Ka'ti.*

Figure A4.20. Manuscript 19, folio 217v and 218r, © *Fondo Ka'ti.*

POEMS OF AL-SHARISHI

Figure A4.21. Poems of Al-Sharishi, folio 1, © *Fondo Ka'ti.*

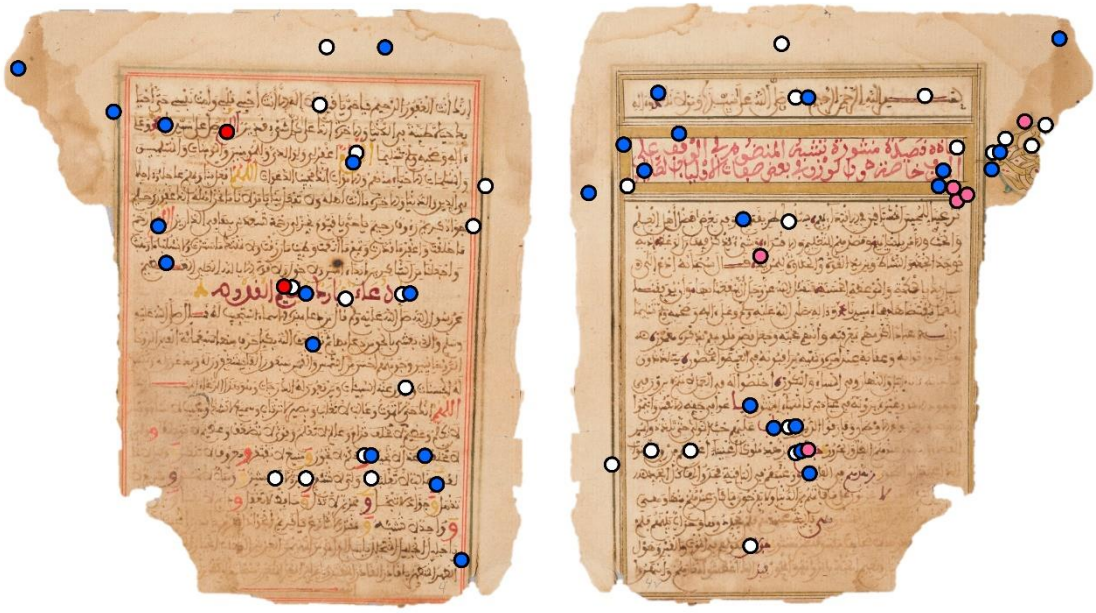


Figure A4.22. Poems of Al-Sharishi, folio 4, © *Fondo Ka'ti.*

Figure A4.23. Poems of Al-Sharishi, folio 10, © *Fondo Ka'ti.*